PCT/EP2004/010161

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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

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Der Präsident des Europäischen Patentamts
Im Auftrag

For the President of the European Patent O

Le Président de l'Office européen des brev
p.o.

R C van Dijk

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Demande no:

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Bezeichnung der Erfindung/Title of the invention/Titre de l'invention:
(Falls die Bezeichnung der Erfindung nicht angegeben ist, siehe Beschreibung.
If no title is shown please refer to the description.
Si aucun titre n'est indiqué se referer à la description.)

4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and their analogues as effective compounds against infectious and other diseases

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4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and their analogues as effective compounds against infectious and other diseases.

Description

The present invention relates to 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and their analogues and pharmaceutically acceptable salts thereof, the use of these derivatives for the prophylaxis and/or treatment of mycobacteria-induced infections, opportunistic infections, autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke as well as compositions containing at least one 4,7-dihydro-5H-thieno[2,3c]pyran derivative or analogue thereof and/or pharmaceutically acceptable salts thereof.

Mycobacteria is the cause for a number of severe diseases, among them tuberculosis, leprosy, and mycobacteria-induced meningitis. Tuberculosis is an ancient scourge of human beings, caused by *Mycobacterium tuberculosis*. Although more than three billion people have been inoculated with the vaccine BCG, presently more than 50,000 people die every week of tuberculosis world-wide, and there are estimations that one third of the world's population is infected by *Mycobacterium tuberculosis*. According to a recent report of the World Health Organisation (WHO) on tuberculosis epidemic, distributed via the internet, it is estimated that between the years 2000 and 2020, nearly one billion people will carry tuberculosis bacteria, 200 million people will get sick, and 35 million will die of tuberculosis, if control of the disease and preventive measures are not strengthened. Moreover, it has been reported that 32% of HIV infected individuals die of tuberculosis. The situation has become even more dramatic since a number of *Mycobacterium tuberculosis* strains have shown a multidrug resistance, which cannot be attacked by conventional therapy, e.g. antibiotics. In addition, immune-suppressed people like AIDS patients are often victims of mycobacterial infections leading to a poor prognosis.

There are several reasons why mycobacteria-induced diseases are difficult to cure: First of all, mycobacteria can perform a differentiation process called "dormancy" or "persistency". Dormant mycobacteria are much more resistant against conventional antibacterial drug treatment. Secondly, many of the mycobacteria species have long

replication times, resulting in a slow growth. One consequence thereof is that antimycobacterial drugs need longer medication times compared to the medication of faster growing pathogenic bacteria. Both factors cited above are reasons why a medical treatment of mycobacteria-induced diseases has to last at least for several months. A third factor why conventional antibacterial drug treatment is so difficult with regard to mycobacteria-induced diseases is that these bacteria have a relatively thick cell wall, which is not or almost not permeable for many substances.

The use of 4,5,6,7-tetrahydrobenzo[b]thiophene derivatives in the treatment of mycobacterial infections such as tuberculosis are described in the as yet unpublished PCT patent application PCT/EP03/03697. The compounds described therein have been found to be effective in blocking the activity of mycobacterial protein serine/threonine kinases, particularly protein kinase G (PknG), which have been identified as an essential component involved in the persistence and enhanced survival of pathogenic mycobacterial within a macrophage cell line, and thereby provide a mode for the elimination of mycobacteria.

Additionally, biologically active 4,7-dihydro-5-H-thieno[2,3c]pyran and 4,7-dihydro-5-H-thieno[2,3-c]thiopyran derivatives are described in *Biorg. Med. Chem. Letters* **2002**, *12*, 1897-1900, in which compounds which inhibit TNF-a-production are described, in *J. Med. Chem.* **2002**, *45*, 4443-4459, in which compounds are described which act as protein-tyrosine phosphatase 1B (PTP1B) inhibitors, or in Japanese patent JP 2002308870, in which compounds are described, which act as Staphylococcus aureus inhibitors. Further derivatives are described in *Armyanskii Khimicheskii Zhurnal* **1987**, 40(9), 581-7. These references do not disclose any PkNG inhibitory activity for these compounds.

In WO 01/98290 thiophene derivatives are described as active kinase inhibitors.

One important feature for pharmaceutical active agents in general is that these agents have a high degree of metabolitic stability. It was found that the compounds described in PCT/EP03/03697, while being pharmaceutically active as PkNG inhibitors, left room for further increase of metabolitic stability.

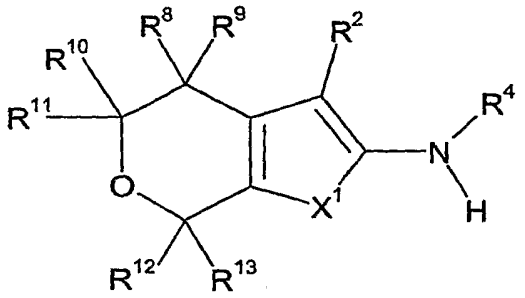
Taking into account the above-mentioned problems with conventional antimycobacterial treatment, it is the object of the present invention to provide compounds and/or pharmaceutically acceptable salts thereof which can be used as pharmaceutically active substances, especially for the prophylaxis and/or treatment of mycobacteria-induced infections, a method to treat mycobacteria-induced

diseases by means of those compounds, as well as compositions comprising at least one of those compounds and/or pharmaceutically acceptable salts thereof as pharmaceutically active ingredients.

A further object is to provide compounds and/or pharmaceutically acceptable salts thereof which can be used as pharmaceutically active substances for the prophylaxis and/or treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.

These objects are solved by the 4,7-Dihydro-5H-thieno[2,3c]pyran derivative and analogous compounds and/or their pharmaceutically acceptable salts of independent claim 1, the compound according to claim 25, the use of at least one of the those compounds and/or the pharmaceutically acceptable salts thereof as pharmaceutically active agents according to independent claim 26, the use of the compounds for the preparation of a medicament for the treatment of various diseases according to independent claims 27 and 36, the use of the compounds as an inhibitor for a protein kinase according to independent claim 45, and the use of at least one compound and/or a pharmaceutically active salt thereof for the preparation of a pharmaceutical composition according to independent claim 51. Further advantageous features, aspects and details of the invention are evident from the dependent claims, the description, the examples and the drawings.

The 4,7-Dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof according to the present invention are represented by the following general formula (I)



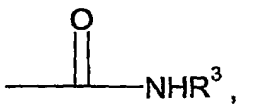
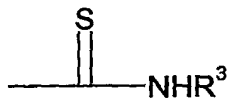
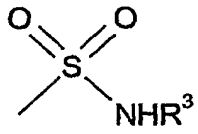
(I)

wherein

X^1 is selected from S, O, NR^1,

and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^2 is selected from

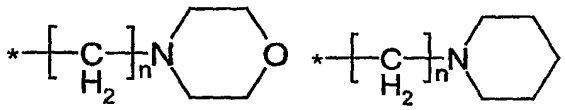




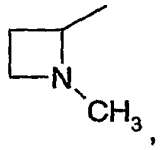
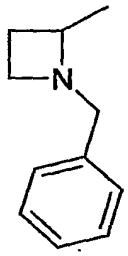
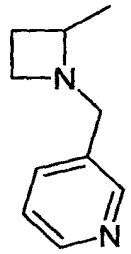
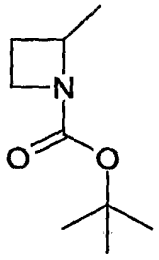
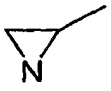
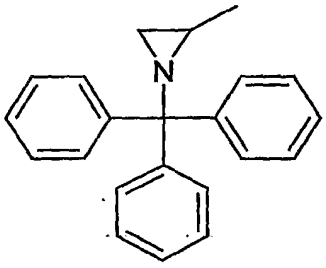
wherein R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_6-alkyl,

R^4 is selected from H , $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, adamantyl,



or $-(CH_2)_n-NR_{14}R_{15}$,

wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted C_1-C_4-alkyl or C_2-C_4-alkenyl and wherein n = 1 to 6,

or NR^6R^7,

wherein

R^6 is selected from H, C_1-C_6-alkyl, and
R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl
R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH
R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl
R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and
R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl,

and include stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

As used in the present invention, the term substituted or unsubstituted C_1-C_6-alkyl or C_1-C_4-alkyl or C_1-C_3-alkyl is meant to include linear or branched alkyls in which optionally one, two or three of the hydrogen atoms bonded to the carbon chain are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl, a -OH or -SH group, a $-NH_2$ group, methoxy or ethoxy group, or phenyl group. These terms therefore especially comprise, depending on the number of carbon atoms in each respective term, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert.-butyl, $-C_5H_{11}$, $-CH_2-C(CH_3)_3$, $-CH(CH_3)-C_3H_7$, $-CH_2-CH(CH_3)-C_2H_5$, $-CH(CH_3)-CH(CH_3)_2$, $-C(CH_3)_2-C_2H_5$, $-CH_2-C(CH_3)_3$, $-C_2H_4-CH(CH_3)_2$, $-C_6H_{13}$, $-C_3H_6-CH(CH_3)_2$, $-C_2H_4-CH(CH_3)-C_2H_5$, $-CH(CH_3)-C_4H_9$, $-CH_2-CH(CH_3)-C_3H_7$, $-CH(CH_3)-CH_2-CH(CH_3)_2$, $-CH(CH_3)-CH(CH_3)-C_2H_5$, $-CH_2-CH(CH_3)-CH(CH_3)_2$, $-CH_2-C(CH_3)_2-C_2H_5$, $-C(CH_3)_2-C_3H_7$, $-C(CH_3)_2-CH(CH_3)_2$, $-C_2H_4-C(CH_3)_3$, $-CH(CH_3)-C(CH_3)_3$, optionally substituted in the above described manner, especially to give phenyl substituted alkyles such as benzyl.

Similarly, the term substituted or unsubstituted C_3-C_6-cycloalkyl is meant to include cyclolalkanes in which optionally one, two or three of the hydrogen atoms bonded to the carbon atoms of the cycle are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl, a -OH or -SH group, a $-NH_2$, methoxy or ethoxy or methyl, ethyl or phenyl group. This term therefore includes cyclopropyl, cyclobutyl,

cyclopentyl, cyclohexyl as well as methyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, ethyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or phenyl substituted cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, optionally substituted in the above described manner.

Similarly, the term unsubstituted or substituted C_2-C_4-alkenyl is meant to include branched or linear alkenyles in which optionally one, two, three or four of the hydrogen atoms bonded to the carbon atoms of the alkyl are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl. These terms therefore are meant to comprise ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl , but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, optionally substituted in the above described manner.

Similarly, the term unsubstituted or substituted C_2-C_4-alkinyl is meant to include branced or linear alkinyles in which optionally one, two, three or four of the hydrogen atoms bonded to the carbon atoms of the alkyl are substituted by a halogen atom such as F, Cl , Br, or I, preferably F or Cl. These terms therefore are meant to comprise prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, and but-3-inyl, optionally substituted in the described above manner.

The term substituted or unsubstituted aryl is meant to include aromatic compounds, in which one, two or three of the hydrogen atoms bonded to the aromatic ring are substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by -NO_2, -OH, -SH, -NH_2, -CN, methyl, acetyl or methoxy. This term is therefore meant to comprise phenyl, 2,3-halogen substituted phenyl, 3,4-halogen substituted phenyl, as well as, for instance, 4-acetylphenyl, 4-methylphenyl or 4-fluorophenyl.

The term substituted or unsubstituted heteroaryl is meant to include aromatic groups in which the aromatic ring comprises at least one heteroatom selected from the group N, O, or S, and in which one, two or three of the hydrogen atoms bonded to the aromatic ring are optionally substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by -NO_2, -OH, -SH, methyl or methoxy. This term therefore includes furanyl, pyrollyl, thienyl, and pyridinyl which optionally can be substituted in the above described manner.

The term substituted or unsubstituted heterocycloalkyl is meant to include cycloalkyles in which at least one of the carbon atoms of the ring, preferably 1 or 2 atoms, have been substituted by a heteroatom selected from the group consisting of N, O, and S which optionally and in which one, two or three of the hydrogen atoms

bonded to the ring are substituted by an halogen, such as F, Cl, Br or I, preferably F and Cl, or substituted by methyl or methoxy. This term therefore includes pyrrolidinyl; piperidinyl and tetrahydrofuranyl, which optionally can be substituted in the above described manner.

In a preferred embodiment of the present invention X^1 is S.

In a further preferred embodiment of the present invention X^1 is NR^1, and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl, and preferably is methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, or benzyl.

In a further preferred embodiment of the present invention X^1 is O.

In a further preferred embodiment of the present invention R^2 is $-C(=O)NHR^3$ and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

In a further preferred embodiment of the present invention R^2 is $-C(=S)NHR^3$ and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

In a further preferred embodiment of the present invention R^2 is $-SO_2NHR^3$ and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

In yet another preferred embodiment of the invention R^3 is selected from the group consisting of H, $-CH_2-CH_2-OH$, $-CH_2-CH_2-NH_2$, $-CH_2-CH_2-SH$, $-CH_2-CH(OH)-CH_3$, $-CH_2-CH(SH)-CH_3$, or $-CH_2-CH(NH_2)-CH_3$.

In a further preferred embodiment of the present invention R^4 is $-C(=X^2)R^5$ and X^2 is O or S, and preferably O.

In a further preferred embodiment of the present invention R^4 is $-SO_2-R^5$.

In yet another preferred embodiment of the invention R_5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, prop-1-enyl, prop-2-enyl , but-1-enyl, but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl, adamantyl, or NR^6R^7, wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl.

In yet another preferred embodiment of the invention R_5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, prop-1-enyl, prop-2-enyl , but-1-enyl, but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl, or adamantyl.

In yet another preferred embodiment of the present invention R_5 is selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, methyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, carboxyl substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, furanyl, methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert.-butyl, prop-1-enyl, but-1-enyl, adamantyl, 3,4-difluorophenyl or NR^6R^7, wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^7 preferably is selected from the group consisting of substituted or unsubstituted C_3-C_6-cycloalkyl, preferably cyclohexyl, or an unsubstituted or substituted phenyl. In a further preferred embodiment of the present invention, R^7 is selected from the group consisting of mono-, di- or tri-substituted phenyl groups, wherein the substituents are selected from the group consisting halogen, such as F, Cl, Br or I, preferably F and Cl, or $-NO_2$, -OH, -SH, $-NH_2$, -CN, C_1-C_6-alkyl, preferably methyl, acyl, preferably acetyl, or methoxy. In a further embodiment of the present

invention the group R^7 is selected from the group consisting of phenyl, 3,4-difluorophenyl, 4-acetylphenyl, or 4-methylphenyl.

In another preferred embodiment of the present invention R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl. In a further embodiment of the present invention, the compound 5,5-dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide is excluded from the compounds according to the present invention. In another embodiment of the present invention, when R^7 is any one of the groups as outlined above, at least one of the groups R^{10} and R^{11} is not methyl and preferably are one or both of these groups is hydrogen.

In yet another embodiment of the present invention R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkoxy, or OH.

In yet another preferred embodiment of the present invention R^8 is H and R^9 is selected from H, or substituted or unsubstituted C_1-C_6-alkyl.

In a further preferred embodiment of the present invention R^8 and R^9 are both H.

In a further preferred embodiment of the present invention R^{10}, R^{11}, R^{12}, and R^{13} are independently selected from H and substituted or unsubstituted C_1-C_6-alkyl, and preferably from H or methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl or tert.-butyl. In yet another preferred embodiment of the present invention R^{10} and R^{11} are methyl and R^{12} and R^{13} are H, or R^{10}, R^{11}, R^{12}, and R^{13} are H, or R^{10}, R^{11}, R^{12}, and R^{13} are methyl, or R^{10} and R^{11} are H and R^{12} and R^{13} are methyl.

In yet another preferred embodiment of the present invention R^{10} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{11} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

In yet another preferred embodiment of the present invention R^{12} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

In a further preferred embodiment of the present invention R^1 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, iso-butyl, tert.-butyl or benzyl.

In a further preferred embodiment of the present invention R_{14} and R_{15} are independently selected from methyl, ethyl and propyl or allyl, and preferably are methyl.

In yet another preferred embodiment of the invention compound according to formula (I) is selected from the group consisting of:

(Compound 1) 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 2) 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 3) 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 4) 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 5) 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 6) 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 7) 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 8) 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 9) 2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 10) 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 11) 2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 12) 2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 13) 2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 14) 2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 15) 2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 16) 5,5-Dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 17) 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide,

(Compound 18) 2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 19) 2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 20) 2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide,

(Compound 21) 2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide, and

(Compound 22) 2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide

The present invention also comprises pharmaceutically active salts of these compounds, all stereoisomeric forms and regioisomeric forms of these compounds or prodrugs thereof.

Other aspects of the present invention relate to the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof as outlined above in the general formula (I) for use as new pharmaceutically active agents, particularly for the prophylaxis and/or treatment of virally or bacterially induced diseases or infections, especially infections induced by bacteria of the genus legionella, and especially legionnaires disease, or mycobateria-induced infections (including opportunistic infections) and diseases, especially mycobacteria induced meningitis, tuberculosis and leprosy,

pharmaceutical compositions comprising these 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof as active ingredients and a method for treating virally and/or bacterially induced diseases, particularly mycobacteria-induced infections, in mammals, including humans, especially for the treatment of treatment of virally or bacterially induced diseases or infections, especially infections induced by bacteria of the genus legionella, and especially legionnaires disease, or mycobateria-induced infections (including opportunistic infections) and diseases, especially mycobacteria induced meningitis, tuberculosis and leprosy.

Other diseases which can be successfully treated with the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and analogues thereof according to the present invention are autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke. What is said above and in the following with regard to the treatment of diseases equally applies with respect to the prophylaxis against respective diseases.

Autoimmune diseases, which may be treated with the compounds of the present invention, are e.g. asthma, chronic obstructive pulmonary diseases, systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, osteoporisis, psoriasis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, multiple sclerosis, alopecia or autoimmune diabetes mellitus.

The same applies not only with respect to the treatment, but also with regard to the prophylaxis against respective diseases.

Cardiovascular diseases which may be treated with the compounds of the present invention, are e.g. adult congenital heart disease, aneurysms, angina, angina pectoris, arrhythmias, cardiovascular disease prevention, cardiomyopathies, congestive heart failure, myocardial infarction, pulmonary hypertension, hypertrophic growth, restenosis, stenosis or arteriosclerosis.

A typical cell proliferative disease which can be treated with the compounds of the present invention is cancer, e.g. bladder cancer, breast cancer, cancer of the central nervous system, cancer of the colon, gastric cancer, lung cancer, kidney cancer, melanoma, head and neck cancer, ovarian cancer, cervix cancer, glioblastoma, pancreas cancer, prostate cancer, stomach cancer, skin cancer, testis cancer, leukaemia, Hodgkin's lymphoma, liver cancer and renal cancer.

The diabetes which can be treated with the compounds of the present invention is diabetes Type I and Type II.

The inflammation which can be treated with the compounds of the present invention may be mediated by cytokines, such as TNF-α, IL-1ß, GM-CSF, IL-6 and/or IL-8.

Among the neurodegenerative diseases which can be treated with the compounds of the present invention are Alzheimer's disease, Parkinson's disease, AIDS-related dementia, Huntington's disease, amyotrophic lateral scelerosis, retinitis pigmentosa, spinal muscular atrophy and cerebrellar degeneration.

Surprisingly, it was found that the compounds according to the present invention as well as pharmaceutically acceptable salts of these derivatives are effective against virally and/or bacterially induced diseases, especially mycobacteria-induced infections and diseases, as well as autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke at pharmaceutically acceptable concentrations while exhibiting enhanced metabolitic stability.

Additionally, the present invention relates to the use of the compounds of the present invention for the manufacturing of a pharmaceutical composition for the prophylaxis and/or treatment of virally and/or bacterially induced diseases, particularly those infections and diseases mentioned above, as well as autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.

The compounds of the present invention are effective against mycobacteria induced infections, particularly tuberculosis, but also e.g. leprosy and mycobacteria-induced meningitis. Mycobacteria which induce or cause these infectious diseases are members of the group comprising the tuberculous bacteria *Mycobacterium tuberculosis*, *M. bovis*, *M. africanum* and *M. leprae* as well as the non-tuberculous bacteria *M. abscessus*, *M. avium*, *M. celatum*, *M. chelonae*, *M. fortuitum*, *M. genavense*, *M. gordonae*, *M. haemophilum*, *M. intracellulare*, *M. kansii*, *M. malmoense*, *M. marinum*, *M. scrofulaceum*, *M. simiae*, *M. szulgai*, *M. ulcerans* and *M. xenopi*. Because of the outstanding clinical importance of tuberculosis, microbiologists have distinguished the so-called "Mycobacterium tuberculosis complex" consisting of *Mycobacterium tuberculosis*, *M. bovis*, and *M. africanum* from all other mycobacteria which form the group of the so-called "atypical mycobacteria" or "non-tuberculous mycobacteria (NTM)".

The present invention also provides a method for preventing or treating infections and diseases, especially virally or bacterially induced diseases or infections, more specially infections induced by bacteria of the genus legionella such as legionaires disease, mycobacteria-induced infections (including opportunistic infections) in mammals (including humans), as well as a method for preventing against and treating diseases, like autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke, which method comprises administering to the mammal an pharmaceutically effective amount of the compounds of the present invention to treat an infection or disease. Especially, the method is used for the treatment of tuberculosis, but also for other mycobacteria-induced infections like leprosy or mycobacteria-induced meningitis.

According to a still further aspect, the present invention refers to pharmaceutical compositions comprising at least one compound according to the present invention as an active ingredient together with at least one pharmaceutically acceptable (i.e. non-toxic) carrier, excipient and/or diluent. The pharmaceutical compositions of the present invention can be prepared in a conventional solid or liquid carrier or diluent and a conventional pharmaceutically-made adjuvant at suitable dosage level in a known way. The preferred preparations are adapted for oral application. These administration forms include, for example, pills, tablets, film tablets, coated tablets, capsules, powders and deposits.

Furthermore, the present invention also includes pharmaceutical preparations for parenteral application, including dermal, intradermal, intragastral, intracutan; intravasal, intravenous, intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan, rectal, subcutaneous, sublingual, topical, or transdermal application, which preparations in addition to typical vehicles and/or diluents contain at least one compound according to the present invention and/or a pharmaceutical acceptable salt thereof as active ingredient.

The pharmaceutical compositions according to the present invention containing at least one compound according to the present invention, i.e. one 4,7-Dihydro-5H-thieno[2,3c]pyran derivative or analogues compound thereof as set out in general formula (I) in independent claim 1 or claims dependent thereon, and/or a pharmaceutical acceptable salt thereof as active ingredient will typically be administered together with suitable carrier materials selected with respect to the intended form of administration, i.e. for oral administration in the form of tablets,

capsules (either solid filled, semi-solid filled or liquid filled), powders for constitution, gels, elixirs, dispersable granules, syrups, suspensions, and the like, and consistent with conventional pharmaceutical practices. For example, for oral administration in the form of tablets or capsules, the active drug component may be combined with any oral non-toxic pharmaceutically acceptable carrier, preferably with an inert carrier like lactose, starch, sucrose, cellulose, magnesium stearate, dicalcium phosphate, calcium sulfate, talc, mannitol, ethyl alcohol (liquid filled capsules) and the like. Moreover, suitable binders, lubricants, disintegrating agents and coloring agents may also be incorporated into the tablet or capsule. Powders and tablets may contain about 5 to about 95 weight % of the 4,7-dihydro-5H-thieno[2,3c]pyran derivative or analogues compound thereof or the respective pharmaceutically active salt as active ingredient.

Suitable binders include starch, gelatin, natural sugars, corn sweeteners, natural and synthetic gums such as acacia, sodium alginate, carboxymethylcellulose, polyethylene glycol and waxes. Among suitable lubricants there may be mentioned boric acid, sodium benzoate, sodium acetate, sodium chloride, and the like. Suitable disintegrants include starch, methylcellulose, guar gum, and the like. Sweetening and flavoring agents as well as preservatives may also be included, where appropriate. The disintegrants, diluents, lubricants, binders etc. are discussed in more detail below.

Moreover, the pharmaceutical compositions of the present invention may be formulated in sustained release form to provide the rate controlled release of any one or more of the components or active ingredients to optimise the therapeutic effect(s), e.g. antihistaminic activity and the like. Suitable dosage forms for sustained release include tablets having layers of varying disintegration rates or controlled release polymeric matrices impregnated with the active components and shaped in tablet form or capsules containing such impregnated or encapsulated porous polymeric matrices.

Liquid form preparations include solutions, suspensions, and emulsions. As an example, there may be mentioned water or water/propylene glycol solutions for parenteral injections or addition of sweeteners and opacifiers for oral solutions, suspensions, and emulsions. Liquid form preparations may also include solutions for intranasal administration.

Aerosol preparations suitable for inhalation may include solutions and solids in powder form, which may be present in combination with a pharmaceutically acceptable carrier such as an inert, compressed gas, e.g. nitrogen.

For preparing suppositories, a low melting wax, such as a mixture of fatty acid glycerides like cocoa butter is melted first, and the active ingredient is then dispersed homogeneously therein e.g. by stirring. The molten, homogeneous mixture is then poured into conveniently sized moulds, allowed to cool, and thereby solidified.

Also included are solid form preparations which are intended to be converted, shortly before use, to liquid form preparations for either oral or parenteral administration. Such liquid forms include solutions, suspensions, and emulsions.

The compounds according to the present invention may also be delivered transdermally. The transdermal compositions may have the form of a cream, a lotion, an aerosol and/or an emulsion and may be included in a transdermal patch of the matrix or reservoir type as is known in the art for this purpose.

The term capsule as recited herein refers to a specific container or enclosure made e.g. of methyl cellulose, polyvinyl alcohols, or denatured gelatins or starch for holding or containing compositions comprising the active ingredient(s). Capsules with hard shells are typically made of blended of relatively high gel strength gelatins from bones or pork skin. The capsule itself may contain small amounts of dyes, opaquing agents, plasticisers and/or preservatives.

Under tablet a compressed or moulded solid dosage form is understood which comprises the active ingredients with suitable diluents. The tablet may be prepared by compression of mixtures or granulations obtained by wet granulation, dry granulation, or by compaction well known to a person of ordinary skill in the art.

Oral gels refer to the active ingredients dispersed or solubilised in a hydrophilic semi-solid matrix.

Powders for constitution refers to powder blends containing the active ingredients and suitable diluents which can be suspended e.g. in water or in juice.

Suitable diluents are substances that usually make up the major portion of the composition or dosage form. Suitable diluents include sugars such as lactose, sucrose, mannitol, and sorbitol, starches derived from wheat, corn rice, and potato,

and celluloses such as microcrystalline cellulose. The amount of diluent in the composition can range from about 5 to about 95 % by weight of the total composition, preferably from about 25 to about 75 weight %, and more preferably from about 30 to about 60 weight %.

The term disintegrants refers to materials added to the composition to support break apart (disintegrate) and release the pharmaceutically active ingredients of a medicament. Suitable disintegrants include starches, "cold water soluble" modified starches such as sodium carboxymethyl starch, natural and synthetic gums such as locust bean, karaya, guar, tragacanth and agar, cellulose derivatives such as methylcellulose and sodium carboxymethylcellulose, microcrystalline celluloses, and cross-linked microcrystalline celluloses such as sodium croscaramellose, alginates such as alginic acid and sodium alginate, clays such as bentonites, and effervescent mixtures. The amount of disintegrant in the composition may range from about 2 to about 20 weight % of the composition, more preferably from about 5 to about 10 weight %.

Binders are substances which bind or "glue" together powder particles and make them cohesive by forming granules, thus serving as the "adhesive" in the formulation. Binders add cohesive strength already available in the diluent or bulking agent. Suitable binders include sugars such as sucrose, starches derived from wheat corn rice and potato, natural gums such as acacia, gelatin and tragacanth, derivatives of seaweed such as alginic acid, sodium alginate and ammonium calcium alginate, cellulose materials such as methylcellulose, sodium carboxymethylcellulose and hydroxypropylmethylcellulose, polyvinylpyrrolidone, and inorganic compounds such as magnesium aluminum silicate. The amount of binder in the composition may range from about 2 to about 20 weight % of the composition, preferably from about 3 to about 10 weight %, and more preferably from about 3 to about 6 weight %.

Lubricants refer to a class of substances which are added to the dosage form to enable the tablet granules etc. after being compressed to release from the mould or die by reducing friction or wear. Suitable lubricants include metallic stearates such as magnesium stearate, calcium stearate, or potassium stearate, stearic acid, high melting point waxes, and other water soluble lubricants such as sodium chloride, sodium benzoate, sodium acetate, sodium oleate, polyethylene glycols and D,L-leucine. Lubricants are usually added at the very last step before compression, since they must be present at the surface of the granules. The amount of lubricant in the composition may range from about 0.2 to about 5 weight % of the composition,

preferably from about 0.5 to about 2 weight %, and more preferably from about 0.3 to about 1.5 weight % of the composition.

Glidents are materials that prevent caking of the components of the pharmaceutical composition and improve the flow characteristics of granulate so that flow is smooth and uniform. Suitable glidents include silicon dioxide and talc. The amount of glident in the composition may range from about 0.1 to about 5 weight % of the final composition, preferably from about 0.5 to about 2 weight %.

Coloring agents are excipients that provide coloration to the composition or the dosage form. Such excipients can include food grade dyes adsorbed onto a suitable adsorbent such as clay or aluminum oxide. The amount of the coloring agent may vary from about 0.1 to about 5 weight % of the composition, preferably from about 0.1 to about 1 weight %.

To identify substances for drug development against mycobacteria-induced diseases, it was searched for inhibitors of signal transduction components present in mycobacteria. As already mentioned above, the elimination of mycobacteria from the human body is presently achieved by inhibiting the growth of respective bacteria by means of antibiotics. According to the present invention, a novel strategy has been used to fight against mycobacteria, namely to attack mycobacterial signal transduction components which are involved in the persistence of the bacteria within the host cell. Previously, it had been shown that mycobacteria penetrate cells via the endocytotic pathway. Endosomes containing non-pathogenic mycobacteria fuse to lysosomes and subsequently the bacteria are degraded by lysosomal enzymes. However, pathogenic mycobacteria, like *Mycobacterium tuberculosis*, contain additional "virulence genes" which prevent fusion of endosomes and lysosomes and thus circumvent the degradation within a host cell.

Mycobacterial protein serine/threonine kinases, particularly protein kinase G (PknG), have been identified as an essential component involved in the persistence and enhanced survival of pathogenic mycobacteria within a macrophage cell line. Furthermore, it could be demonstrated that the activity of PknG is an essential factor for virulence of mycobacteria. In accordance with the present invention, compounds have been found which are blocking the activity of PknG in a submicromolar range thus showing that PknG is a suitable target for recognising diseases, monitoring diseases, and controlling therapy of diseases related to mycobacterial infections. These compounds (inhibitors) were able to induce efficient degradation of

mycobacteria within host cells so that the present invention provides a novel mode for elimination of mycobacteria.

With the compounds according to the present invention, besides protein kinases, the activity of further proteins and enzymes, respectively, can be influenced. Such further proteins and enzymes are e.g. nucleotide binding proteins, ATP-binding proteins,, and kinases, such as lipid kinases. The currently known protein kinases which can be affected with the compounds of the present invention are shown in Table III at the end of the specification.

It has been found that certain disease inducing factors can be secreted by a cellular organism to the environment of the organism. Specifically, in the present case it has been found that mycobacterial proteins are secreted from the bacterium *Mycobacterium tuberculosis* to the environment of such a bacterium. A protein, which can be secreted by *Mycobacterium tuberculosis* is the protein serine/threonine kinase PknG. The fact that the above-mentioned inventive compounds are particularly effective against PknG may be due to the fact that this protein kinase can be attacked by these compounds without the need to penetrate the (thick) cell wall of *Mycobacterium tuberculosis*.

The compounds according to the present invention are obtainable by different synthetic routes. One route, which leads to 4,7-dihydro-5H-thieno[2,3c]pyran derivatives starts with the reaction of tetrahydro-pyran-4-one or a correspondingly substituted derivative thereof with an cyano-actetate ester under acidic or basic conditions, preferably under acidic conditions, and under elimination of water and subsequent reaction of the reaction product with sulfur in the presence of an organic base to give a corresponding 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative.

As a next step, the amino group in the thus obtained 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative can be acylated to give a corresponding 2-carbonylamino compound. As an acylation reagent a carboxylic acid chloride is preferably used. This reaction can optionally be carried out in the presence of a base such as an tertiary amide, preferably $NEt(^{i}Pr)_2$.

Other suitable reactions to obtain the secondary carboxylic acid amides can be used, for instance reaction of the amino group with a carboxylic acid and a coupling-agent as used in peptide chemistry, such as HOBT,HOOBT,HBTU or HOAt.

Alternatively, if instead of the acyl group a sulfonyl group is to be attached to the amino group in 2-position, the 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester derivative can be reacted with a sulfonyl chloride compound to give a corresponding 2-sulfanylamino derivative.

The thus obtained compounds can then optionally be reacted with bromine in the presence of an organic acid, preferably acetic acid, to substitute one hydrogen in 7-position of the heterocyclic nucleus by a hydroxyl group.

The above described 3-carboxylic acid ester derivative compounds can then be reacted in a subsequent reaction step with an alkali metal amide, such as $LiNH_2$ or $NaNH_2$, in a polar solvent, which is essentially inert to the alkali metal amide, to give the corresponding 3-carboxylic acid amide derivative. This reaction is preferably carried out under the exclusion of moisture and optionally under an inert atmosphere. The application of lithium amide instead of sodium amide results in higher yields and purer products.

To prepare the corresponding 4,7-dihydro-5H-thieno[2,3-c]pyran derivatives in which a sulfonamide is attached in 3-position, in a first step, 4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-2-amine can be acylated, preferably using a carboxylic acid chloride to give the corresponding 2-carbonyl-amino derivative. This compound can then be reacted with sulfurylchloride, preferably under an inert atmosphere and subsequently with ammonia to give the 3-sulfonamide compound.

If the compounds used to synthesise the compounds according to the present invention contain -NH, -SH or -OH functional groups which potentially interfere with the desired reaction, these may of course be protected with suitable protective groups, which can later on be removed from the respective compounds.

To obtain those analogues of the 4,7-dihydro-5H-thieno[2,3c]pyran derivatives in which the S-atom in the 5-membered ring of the heterocyclic nucleus is substituted either by NR^1 or O, the following synthetic approach can be utilized, which is partially based on a method described in Hauser, C.R., Hoffenberg, D.S.; *J.Org.Chem.* **1955**, *20*, 1448 - 1453.

To obtain the O-analogue compounds the amino group in 2-position a corresponding 2-Amino-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane derivatives can be acylated in a first reaction step, using the acylation reaction described above with reference to the acylation of the 2-amino-4,7-dihydro-5H-thieno[2,3c]pyran-3-carboxylic acid ester

derivatives, i.e. preferably using a carboxylic acid chloride as an acylation agent, obtionally in the presence of a tertiary amine base such as $NEt(^iPr)_2$.

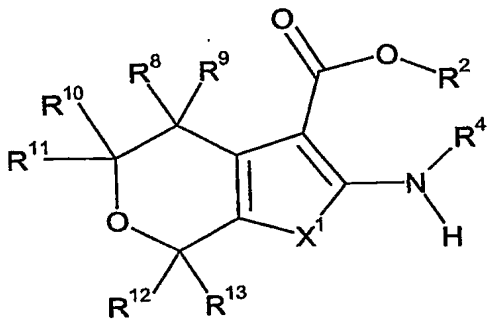
Similarly, to obtain the NR^1-analogue compounds, a corresponding 2-Amino-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane derivative is acylated in the above described manner.

The respective 2-carbonyl-amino derivatives obtained by this acylation can then be reacted with boron triflouride-acetic acid complex $[BF_3 \cdot (HOAc)_2]$ and subsequently treated with an aqueous alkali metal hydroxide solution, such as sodium hydroxide, to convert the cyano group in 3-position of the heterocyclic nucleus into the carboxamide group.

In a further aspect of the present invention, the invention is directed at a method for amidation of an carboxylic acid ester to give the corresponding primary carboxylic acid amide. This amidation comprises the step of reacting an carboxylic acid ester with an alkali metal amide in the presence of a polar solvent, which is essentially inert against the alkali metal amide. Preferably, the molar ratio of carboxylic acid ester to alkali metal amide lies in the range of 1:1 to 1: 15., more preferably in the range of 1:5 to 1:13 and most preferably in the range of 1:9 to 1: 13.

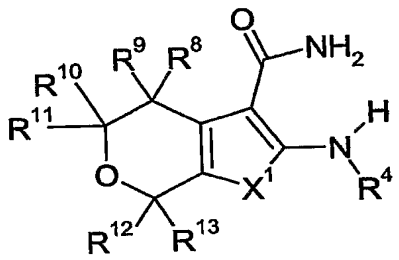
In a preferred embodiment of the method of the present invention, the alkali metal amide is $LiNH_2$ or $NaNH_2$, and preferably is $LiNH_2$. The solvent is preferably absolute ether or absolute tetrahydrofurane, preferably tetrahydrofurane, and the reaction is preferably carried out under the exclusion of moisture. Preferably, the reaction is carried out at a temperature of 15°C to 35°C, preferably at 25°C. It is furthermore preferred that the reaction duration lies in the range of from 40 to 80 hours, preferably from 45 to 75 hours.

In a preferred embodiment of the method of the present invention, the carboxylic acid ester is a compound according to the following general formula (D):



(D)

which is amidated to give the primary carboxylic acid amide according to formula (E),



(E)

wherein in formulas (D) and (E)

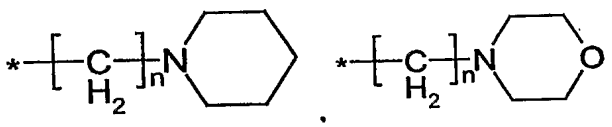
X^1 is selected from S, O, or NR^1, and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

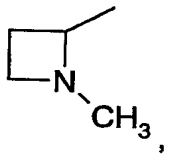
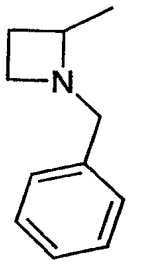
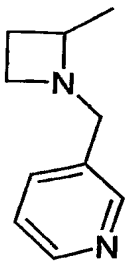
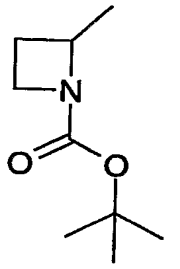
R^2 is linear or branched C_1-C_6 alkyl or aryl and preferably is methyl, ethyl, phenyl or benzyl,

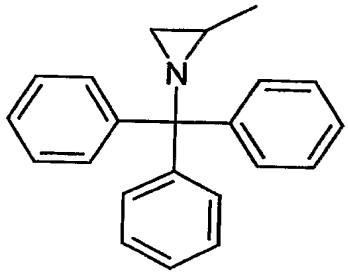
R^4 is selected from H , $-C(=X^2)R^5$ and $-SO_2R^5$,

wherein X^2 is O, S or NH and

R^5 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, adamantyl,





or $-(CH_2)_n-NR_{14}R_{15}$,

wherein R_{14} and R_{15} are independently selected from substituted or unsubstituted C_1-C_4-alkyl or C_2-C_4-alkenyl and wherein n = 1 to 6,

or NR^6R^7,

wherein

R^6 is selected from H, C_1-C_6-alkyl, and

R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl

R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH

R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl

R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl,

and stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

In a further preferred embodiment of the method of the present invention, in general formulas (D) and (E)

X^1 is S

R^2 is methyl or ethyl,

R^4 is -C(=O)R^5 and R5 is selected from methyl, ethyl, propyl, butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH,

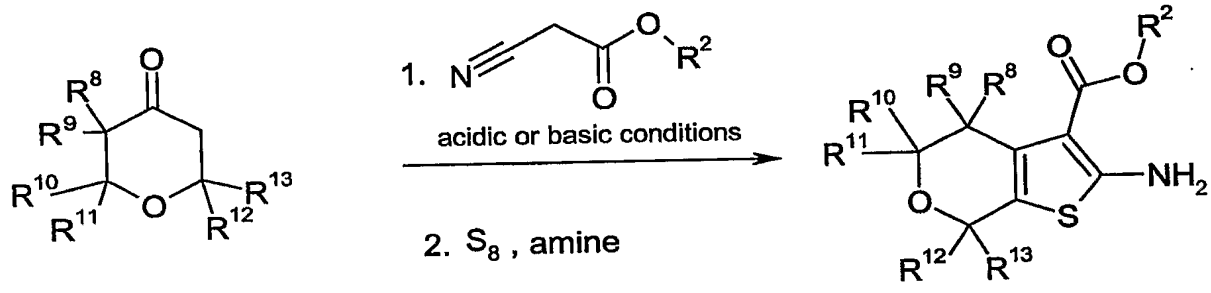
R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl,

R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and

R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

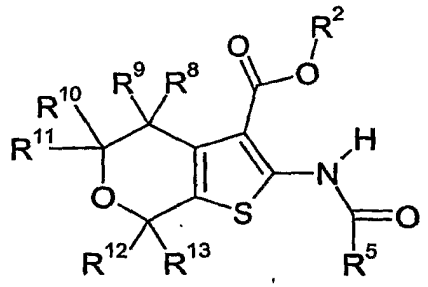
According to one preferred embodiment of the method of the present invention, the compound according to the general formula (E) is obtained by the following reaction sequence:

Step I:



(B) (C)

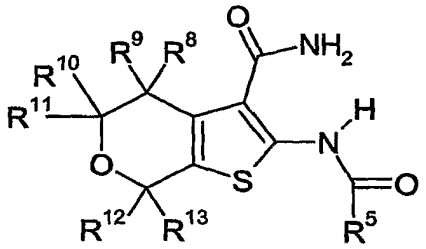
Step II: acylation of the -NH_2 group in 2-position in compound C with $R^5C(=O)LG$, wherein LG represents a suitable leaving group, preferably a halogen such as F, Cl, Br or I, most preferably Cl, to give compound (D):



(D)

and,

Step III: Amidation of as outlined in any one of claims 60 to 65,
to give compound (E):



(E)

It is preferred that in Step I the reaction of compound (B) with the cyano-acetate ester is carried out in a nonpolar solvent, preferably benzene, with the addition of a mixture of ammonium acetate and acetic acid in a molar ratio of greater than 1, preferably in the range from 0.5:1 to 0.8 to 1, and preferably at a temperature in the range of 50 to 100 °C, preferably between 70 to 90 °C, preferably under removal of water formed in the reaction, and preferably for a duration of 2 to 4 hours.

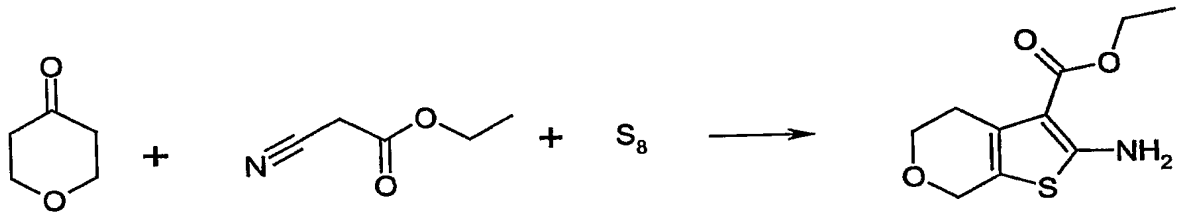
Furthermore, in a preferred embodiment of the present invention, in Step I the reaction product of the reaction of compound (B) with the cyano-acetate ester is reacted with the S_8 in a protic solvent, preferably EtOH, S_8 being added at least in aquimolar quantities, preferably in an excess of up to 1,5, more preferably of up to 1,2, in the presence of a amine base, preferably morpholine, at a reaction temperature of between 25 to 65 °C, preferably between 40 and 60 °C, and preferably for a duration of 2 to 6 hours.

Examples

Chemicals were purchased from Sigma-Aldrich. Waters alliance LC system, equipped with Micromass Quadrupole MS detector was used for the purity analysis. NMR data were measured with a Bruker 300 MHz NMR spectrometer.

Syntheses of compounds

I. Preparation of **Ethyl 2-amino-4,7-dihydro-5*H*-thieno[2,3-*c*]pyran-3-carboxylate**

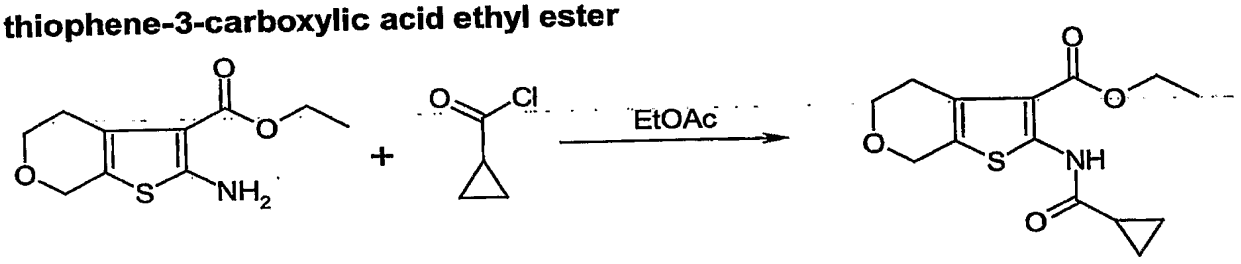


1 mM tetrahydro-pyran-4-one, 106 µL (1 mM) ethyl-cyano-acetate, 0.15 mM ammonium-acetate, and 0.2 mM acetic acid where dissolved in 3 mL benzene and stirred at reflux temperature in a round-bottomed flask equipped with water-remover trap, for 3 hours. The reaction mixture was washed with 2 mL 10% K_2CO_3 solution, dried, and evaporated to dryness. The solid material was dissolved in 1.5 mL EtOH and was stirred with 1.05 mM sulphur and 0.575 mM morpholine at 45-50 °C, for 4 hours. The reaction mixture was evaporated to dryness, washed with n-hexane and isopropylalcohol. This reaction step was developed starting from a procedure described by Gewald, K; Schinke, E; Böttcher, H; *Chem. Ber.* **1966**, *99*, 974.

Yield: 57 %

NMR: 7.28 (s, 2H), 4.43 (s, 2H), 4.16 (q, 2H), 3.79 (t, 2H), 3.67 (t, 2H), 1.25 (t, 3H)

II. Preparation of **2-(Cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b] thiophene-3-carboxylic acid ethyl ester**



1 mM cyclopropanecarbonyl chloride was added dropwise to a well stirred, 15 mL ethylacetate solution of 301 mg (1.00 mM) 2-amino-6-phenyl-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester. The reaction mixture was stirred for

3 hours, then diluted to 50 mL, washed two times with water, dried with $MgSO_4$, and evaporated to dryness. The product was washed with n-hexane and isopropanol. Yield: 42 %

NMR: 11.19 (s, 1H), 4.60 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 2.03 (m, 1H), 1.33 (t, 3H), 0.93 (m, 4H)

Analogous to this method the following compounds were also synthesized:

2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 57%), NMR: 11.90 (s, 1H), 8.06 (s, 1H), 7.39 (d, 1H), 6.79 (dd, 1H), 4.66 (s, 2H), 4.35 (q, 2H), 3.86 (t, 2H), 2.82 (t, 2H), 1.35 (t, 3H);
2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 67%), NMR: 11.36 (s, 1H), 4.62 (s, 2H), 4.32 (q, 2H), 3.84 (t, 2H), 2.79 (t, 2H), 2.06 (bs, 2H), 1.90 (s, 8H), 1.72 (s, 6H), 1.33 (t, 3H);
2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 70%), NMR: 11.10 (s, 1H), 4.61 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 1.90 (d, 2H), 1.69 (m, 3H), 1.43-1.18- (m, 9H);
2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 57%), NMR: 11.15 (s, 1H),4.60 (s, 2H), 4.25 (q, 2H), 3.64 (t, 2H), 2.78 (t, 2H), 1.80 (m, 1H), 1.33 (t, 3H), 1.11 (d, 3H), 0.79 (m, 1H);
2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 76%), NMR: 10.91 (s, 1H), 4.61 (s, 2H), 4.28 (q, 2H),3.83 (t, 2H), 3.44 (m, 1H), 2.78 (bs, 2H), 2.23 (m, 4H), 1.97 (m, 1H), 1.83 (m, 1H), 1.31 (t, 3H);
2-Acetylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 85%), NMR: 10.93 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.77 (t, 2H), 2.24 (s, 3H), 1.32 (t, 3H);
2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 64%), NMR: 10.93 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.77 (t, 2H), 2.24 (s, 3H), 1.32 (t, 3H);
2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 76%), NMR: 11.02 (s, 1H), 6.90 (m, 1H), 6.35 (dd, 1H), 4.63 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.79 (t, 2H), 1.92 (s, 3H), 1.89 (s, 3H), 1.32 (t, 3H);
2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 69%), 11.05(s,1H),4.62(s,2H),4.30(q,2H), 3.84(t,2H), 2.80(t,2H), 2.59(m,1H), 1.64(m,1H), 1.50(m,1H), 1.32(t,3H), 1.14(d,3H), 0.87(t,3H);
2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (yield 76%), NMR: 11.05(s,1H),4.62(s,2H),4.30(q,2H),

3.84(t,2H), 2.80(t,2H), 2.59(m,1H), 1.64(m,1H), 1.50(m,1H), 1.32(t,3H), 1.14(d,3H), 0.87(t,3H);

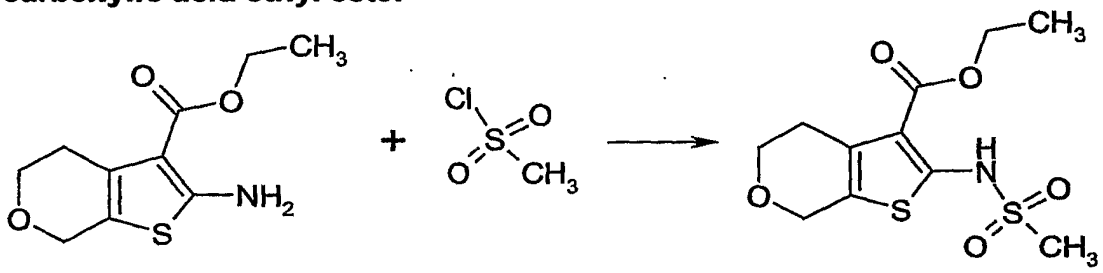
2-(2-Chloro-acetylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 82%), NMR: 11.64 (s, 1H), 4.64 (s, 2H), 4.61 (s, 2H), 4.32 (q, 2H), 3.85 (t, 2H), 2.80 (t, 2H), 1.32 (t, 3H);

2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 79%), NMR: 11.89 (s, 1H), 7.95 (m, 1H), 7.76 (m, 2H), 4.67 (s, 2H), 4.34 (q, 2H), 3.87 (t, 2H), 2.82 (t, 2H), 1.34 (t, 3H);

2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 80%), NMR: 11.08 (s, 1H), 4.62 (s, 2H), 4.30 (q, 2H), 3.84 (t, 2H), 2.78 (m, 3H), 1.32 (t, 3H), 1.17 (d, 6H);

2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester (Yield 77%), NMR: 11.04 (s, 1H), 4.61 (s, 2H), 4.29 (q, 2H), 3.84 (t, 2H), 2.99 (m, 1H), 2.78 (t, 2H), 1.91 (m, 2H), 1.65 (m, 6H), 1.31 (t, 3H).

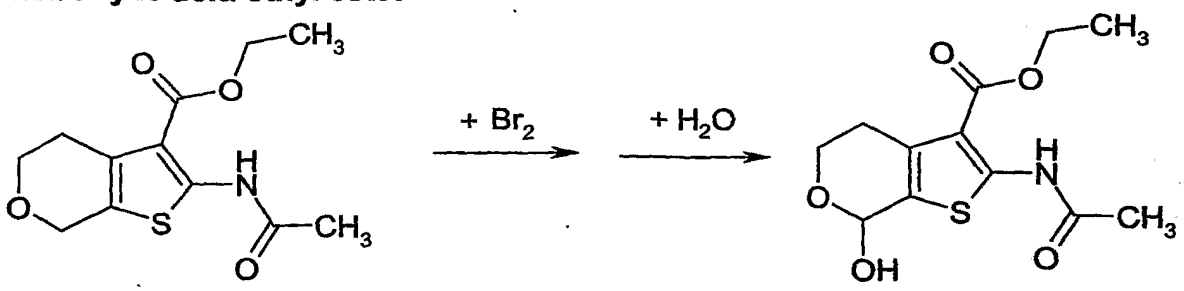
III. Preparation of **2-Methanesulfonylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester**



1 mmol 2-Amino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 10 ml benzene and 348 µL (2.5 equivalent) triethylamine, 195 µL (2.5 equiv.) methanesulfonyl chloride was added. The reaction mixture was refluxed for 8 hours. The mixture was extracted with 1x 15mL water, 1x 15 mL $NaHCO_3$, then 1x 15 ml water, 1x 15 mL 1N HCl and saturated NaCl solution. The organic layer was dried above $MgSO_4$, the solvent was evaporated to vacuo and the residue was crystallized from hexane-isopropanol. (TLC-Eluent: Hexan-Ethylacetate: 2:1)

Yield: 65%, NMR: 11.03 (s, 1H), 4.73 (s, 2H), 4.28 (q, 2H), 3.89 (t, 2H), 3.53 (s, 3H), 2.83 (t, 2H), 1.29 (t, 3H).

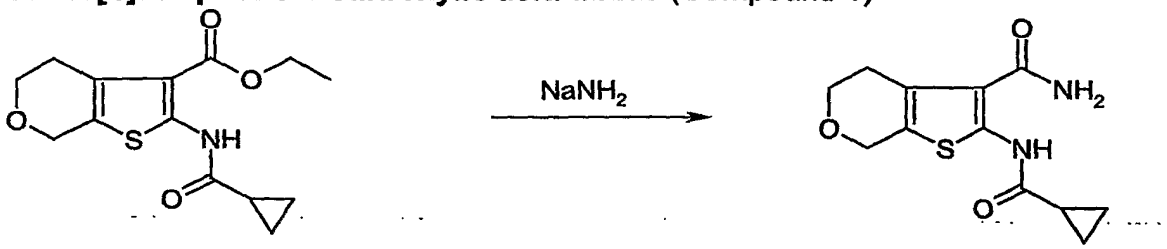
IV. Preparation of **2-Acetamino-7-hydroxy-4,7-dihidro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester**



269 mg (1 mmol) 2-Acetylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 15 mL acetic acid and 82 mg (1 mmol) sodium-acetate was added to the mixture, then heated to 55 ^{0}C. 159 mg bromine in 15 mL acetic acid was added slowly to the mixture. After one hour stirring it was evaporated under reduced pressure and extracted three times with ethyl acetate and 15 mL water. The organic layer was washed with 10 mL $NaHCO_3$ solution and dried with $MgSO_4$. The solution was evaporated under reduced pressure and the product was crystallized from hexane. The product was washed with IPA, and recrystallized with diisopropyl-ether. Yield: 39% NMR: 10.92 (s, 1H), 4.83 (d, 1H), 4.73 (d, 1H), 4.49 (d, 1H), 4.29 (q, 2H), 3.90 (d, 1H), 3.65 (d, 1H), 2.24 (s, 3H), 1.32 (t, 3H).

The compound 2-(Cyclopropanecarbonyl-amino)-7-hydroxy-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was synthesized in a analogous reaction. Yield: 62%, NMR: 11.20 (s,1H), 5.65 (s, 1H), 4.93-4.65 (m, 2H), 4.34 (q, 2H), 4.24-4.03 (m, 3H), 2.10 (m, 1H), 1.38 (t, 3H), 0.93 (m, 4H).

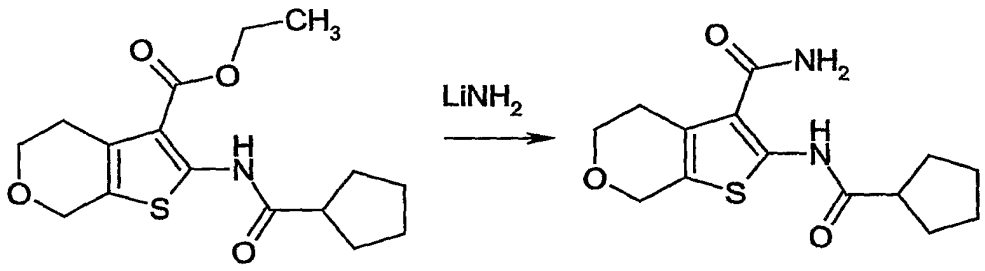
V. Preparation of **2-(Cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid amide** (Compound 1)



470 mg (12.00 mM) sodium amide was added to the solution of 293 mg (1.00 mM) 2-(cyclopropanecarbonyl-amino)-4,5,6,7-tetrahydro-benzo[b]thiophene-3-carboxylic acid ethyl ester in 8 mL abs. tetrahydrofurane. The air-tightly closed reaction mixture was stirred at room temperature for 72 hours.

After the starting material disappeared, the pH of the reaction mixture was set to 5 – 6 with ice cold, 1 N HCl, the precipitated product was filtered off, washed twice with 5 mL n-hexane and dried.
Yield: 89 % white, or off-white crystals; NMR: 11.75 (s, 1H), 4.62 (s, 2H), 3.83 (t, 2h), 2.79 (t, 2H), 1.89 (m, 1H), 0.87 (m, 4H)

VI. Preparation of **2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 2)



1 mmol 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid ethyl ester was dissolved in 3 mL abs. THF, then 230 mg (10 equivalent) $LiNH_2$ was added and the mixture was stirred in a stoppered flask at r.t. for 48 hours. The reaction mixture was poured on ice water, the pH of the solution was adjusted to 5 with 5% HCl. The precipitated crystals were filtered out and washed with cold isopropanol. (TLC Eluent: chloroform-MeOH 10:1)
Yield: 79%, NMR: 11.73 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.88 (m, 1H), 2.80 (t, 2H), 1.89 (m, 2H), 1.64 (m, 6H).

The following compounds were also prepared by this method:
2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3), Yield: 67%, NMR: 11.77 (s, 1H), 7.2 (bs, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 2.46 (m, 1H), 1.60 (m, 1H), 1.47 (m, 1H), 1.12 (d, 3H), 0.85 (t, 3H);
2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 4), Yield: 74%, NMR: 11.63 (s, 1H), 7.2 (bs, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 3.33 (m, 1H), 2.81 (t, 2H), 2.20 (m, 4H), 1.97 (m, 1H), 1.83 (m, 1H);
2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 5), Yield: 73%, NMR: 11.26 (s, 1H), 7.32-7.19 (m, 5H), 4.62 (s, 2H), 4.28 (q, 2H), 3.84 (t, 2H), 2.78 (t, 2H), 1.55 (m, 1H), 1.43 (m, 1H), 1.30 (t, 3H);

2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6), Yield: 49%, NMR: 11.64 (s, 1H), 7.3 (bd, 2H), 6.83 (m, 1H), 6.23 (dd, 1H), 4.64 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 1.89 (d, 3H);

2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7), Yield 31%, NMR: 11.56 (s, 1H), 7.2 (bs, 2H), 5.93 (s, 1H), 4.64 (s, 2H), 3.83 (t, 2H), 2.80 (t, 2H), 2.16 (s, 3H), 1.90 (s, 3H);

2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8), Yield 32%, NMR: 12.33 (s, 1H), 7.2 (bs, 2H), 4.64 (s, 2H), 3.83 (t, 2H), 2.83 (t, 2H), 1.22 (s, 9H);

2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 9), Yield: 70%; NMR: 13.01 (s, 1H), 7.88 (m, 1H), 7.70 (m, 2H), 7.30 (bs, 2H), 4.69 (s, 2H), 3.86 (t, 2H), 2.86 (t, 2H);

2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10), Yield: 61%, NMR: 11.81 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.83 (t, 2H), 2.81 (t, 2H), 2.67 (m, 1H), 1.14 (d, 6H);

2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 11), Yield: 65%, NMR: 11.20 (s,1H), 5.65 (s, 1H), 4.93-4.65 (m, 2H), 4.34 (q, 2H), 4.24-4.03 (m, 3H), 2.10 (m, 1H), 1.38 (t, 3H), 0.93 (m, 4H);

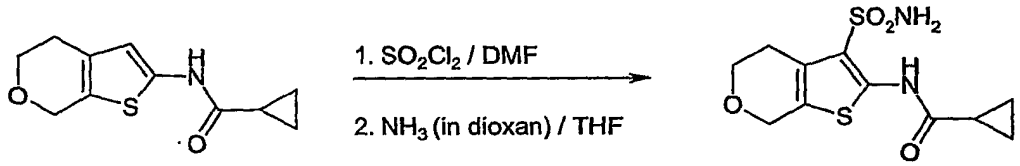
2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 12), Yield: 53%, NMR: 11.71 (s, 1H), 7.5 (bs, 1H), 7.0 (bs, 1H), 4.61 (s, 2H), 3.82 (t, 2H), 2.79 (t, 2H), 1.64 (m, 1H), 1.09 (d, 3H), 0.73 (m, 1H);

2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 13), Yield: 34%, NMR: 12.72 (s, 1H), 8.02 (d, 1H), 7.7 (bs, 1H), 7.31 (d, 1H), 7.2 (bs, 1H), 6.76 (dd, 1H), 4.67 (s, 2H), 3.85 (t, 2H), 2.86 (t, 2H);

2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 14), Yield: 61%, NMR: 12.23 (s, 1H), 7.3 (b, 2H0, 4.63 (s, 2H), 3.83 (t, 2H), 2.83 (t, 2H), 2.03 (s, 3H), 1.86 (s, 5H), 1.70 (s, 5H);

2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 15), Yield: 63%, NMR: 11.79 (s, 1H), 7.2 (bd, 2H), 4.63 (s, 2H), 3.82 (t, 2H), 2.80 (t, 2H), 2.41 (m, 1H), 1.89-1.62 (m, 5H), 1.40-1.17 (m, 5H).

Preparation of **2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide** (Compound 17)



Sulfurylchloride (13 mmol) was added dropwise to DMF (13 mmol) at 0 °C under Argon. The mixture was stirred for 30 min at 0 °C and 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyrane (10 mmol) in 2 ml DCM added. The mixture was stirred for 1 h at r.t., diluted with 2 ml of THF and treated with an excess of NH_3 (2 M solution in dioxane, 10 ml, 20 mmol). The mixture was stirred at room temperature overnight. Evaporation of the solvent and recrystallization afforded the title compound.

Preparation of **2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide** (Compound 19)

0.20 g (1.00 mmol) 2-Amino-4,7-dihydro-5H-thieno[2,3-c]thiopyran-3-carboxylic acid amide and 0.06 g (0.50 mmol) 4-dimethylamino-pyridine was dissolved in 10 cm^3 absolute 1,4-dioxane. The reaction mixture was treated with 0.14 g, 0.13 cm^3 (1.20 mmol) phenylisocyanate in one portion, at room temperature. After stirring for 24 hours at room temperature the solvent was evaporated under reduced pressure. The residue was stirred in the mixture of 15 cm^3 water and 5 cm^3 ethyl acetate for half an hour, at 0 °C. The product was filtered of, washed with 5 cm^3 cold ethyl acetate, and air-dried.
Yield: 0.26 g (82 %)
Mp.: 200-202 °C
Rt: 3.22 min; Mol. Mass: 317
^{1}H NMR DMSO-d6 300MHz, δ(ppm): 10.88 (s, 1H), 10.05 (s, 1H), 7.47 (d, J=8.01 Hz, 2H), 7.35 (broad s, 1H), 7.28 (t, J=7.68 Hz, 2H), 6.99 (t, J=7.32 Hz, 1H), 6.90 (broad s, 1H), 4.62 (m, 2H), 3.83 (m, 2H), 2.80 (m, 2H).

Method II

0.20 g (1.00 mmol) 2-Amino-4,7-dihydro-5H-thieno[2,3-c]thiopyran-3-carboxylic acid amide was dissolved in the mixture of 1 cm^3 absolute pyridine and 1 cm^3 anhydrous DMSO . The mixture was treated with (1 mmol) isocyanate at room temperature. After stirring for 1 hour at room temperature the reaction mixture was treated with cold 1 N aqueous hydrochloric solution and the precipitate was filtered off, then partioned between 1 N aqueous hydrochloric solution and ethyl acetate. The organic

phase was collected, dried and concentrated under reduced pressure. The residue was treated with the mixture of ethyl acetate-hexane and the product was obtained by filtration of the crystalline product.

According to this method the following compounds were prepared:

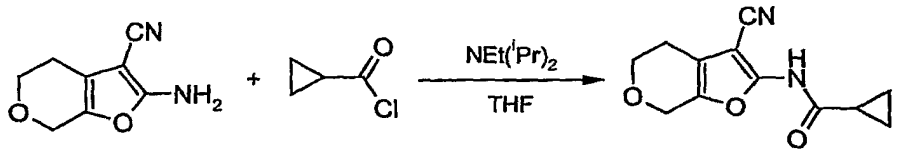
2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 18)
Yield: (32 %)
Mp.: 195.7-196.6 °C
Rt: 1.8 min; Mol. Mass: 323
^{1}H NMR DMSO-d6 300MHz, δ(ppm): 10.22(s, 1H), 10.10(s, 1H), 7.3, 7.0 (b, 2H), 4.62(s, 2H), 3.92(m, 1H), 3.82(t, 2H), 2.78(t, 2H), 2.0-1.42(m, 10H)

2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 20)
Yield: (81 %)
Mp.: 268-269 °C
Rt: 3.10 min; Mol. Mass: 359
^{1}H NMR DMSO-d6 300MHz, δ(ppm): 11.00(s, 1H), 10.44(s, 1H), 7.89(d, 2H), 7.59(d, 2H), 7.4, 6.9(bs, 2H), 4.61(s, 2H), 3.82(t, 2H), 2.78 (t, 2H), 2.48(s, 3H)

2-[3-(4-Methoxy-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 21)
Yield: (81 %)
Mp.: decomposed at 200 °C
Rt: 3.12 min; Mol. Mass: 347
^{1}H NMR DMSO-d6 300MHz, δ(ppm): 10.82(s, 1H), 9.81(s, 1H), 7.36(d, 2H), 6.84(d, 2H), 7.00(b, 2H), 4.59(s, 2H), 3.81(t, 2H), 3.70(s, 3H), 2.77(t, 2H)

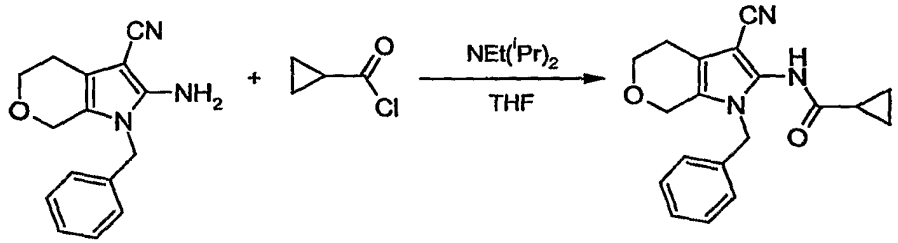
2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 22)
Yield: (80.6 %)
Mp.: decomposed at 280 °C
Rt: 3.34 min; Mol. Mass: 335
^{1}H NMR DMSO-d6 300MHz, δ(ppm): 10.91(s, 1H), 10.08(s, 1H), 7,48(m, 2H), 7.45, 6.95(b, 2H), 7.12(t, 2H), 4,61(s, 2H), 3.82 (t, 2H), 2.79(t, 2H)

Preparation of **2-[(Cyclopropanecarbonyl)-amino]-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane**



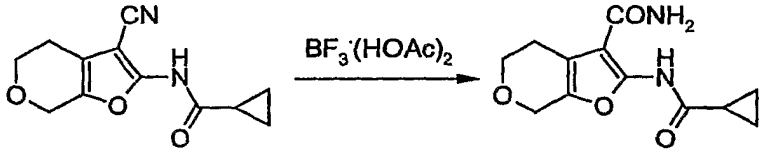
2-Amino-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane (0.66 mmol) and cyclopropylcarbonyl chloride (0.8 mmol) were dissolved in 10 mL of abs. THF. Diisopropylethylamine (0.8 mmol) was added via syringe, and the mixture was stirred overnight at room temperature. After dilution with 20 mL of water, the aqueous phase was extracted four times with ethylacetate, the organic layer washed once with water, dried over sodium sulfate and the solvents evaporated. Recrystallization of the crude material from hot ethanol gave the desired product.

Preparation of **1-Benzyl-2-[(Cyclopropanecarbonyl)-amino]-3-Cyano-4,7-dihydro-5*H*-pyrrolo[2,3-c]pyrane**



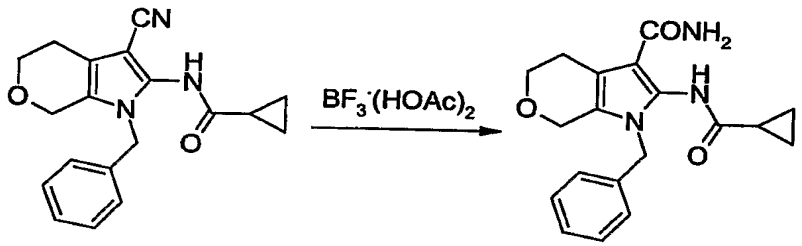
1-Benzyl-2-Amino-3-Cyano-4,7-dihydro-5H-pyrrolo[2,3-c]pyrane (4.1 mmol) and cyclopropylcarbonyl chloride (4.9 mmol) were dissolved in 10 mL of abs. THF. 1.5 mL of diisopropylethylamine were added via syringe, and the mixture was stirred overnight at room temperature. After dilution with 20 mL of water, the aqueous phase was extracted four times with ethylacetate, the organic layer washed once with water, dried over sodium sulfate and the solvents evaporated. Recrystallization of the crude material from hot ethanol gave the desired product.

Preparation of **2-[(Cyclopropanecarbonyl)amino]-4,7-dihydro-5H-furo[2,3-c]pyrane-3-carboxamide**



2-(Cyclopropanecarbonyl-amino)-3-Cyano-4,7-dihydro-5H-furo[2,3-c]pyrane (4.3 mmol), 1 mL of water, and 7 mL of boron trifluoride-acetic acid complex are heated at 120 °C for 10 minutes. After cooling, the reaction mixture is treated with 50 mL of 6 *N* sodium hydroxide solution, the aqueous mixture is extracted with ethylacetate, dried over sodium sulfate and the solvents evaporated. The crude material can be recrystallized from hot ethanol.

Preparation of **1-Benzyl-2-[(Cyclopropanecarbonyl)amino]-4,7-dihydro-5*H*-pyrrolo[2,3-c]pyrane -3-carboxamide**



1-Benzyl-2-(Cyclopropanecarbonyl-amino)-3-Cyano-4,7-dihydro-5*H*-pyrrolo[2,3-c]pyrane (4 mmol), 1 mL of water, and 7 mL of boron trifluoride-acetic acid complex are heated at 120 °C for 10 minutes. After cooling, the reaction mixture is treated with 50 mL of 6 *N* sodium hydroxide solution, the aqueous mixture is extracted with ethylacetate, dried over sodium sulfate and the solvents evaporated. The crude material is recrystallized from hot ethanol.

Biochemical methods and experiments

In the following documents, background information is given with regard to the methods, micoorganisms and enzymes used according to the present invention: Peirs et al., *A serine/threonine protein kinase from Mycobacterium tuberculosis*, Eur. J. Biochem., Mar 1, 244(2), 604-612 (1997); Arruda et al., *Cloning of an M. tuberculosis DNA fragment associated with entry and survival inside cells*, Science 261, 1454-1457 (1993); Wieles et al., *Increased intracellular survival of Mycobacterium smegmatis containing the Mycobacterium leprae thioredoxin-thioredoxin reductase gene*, Infect Immun. 65(7), 2537-2541 (1997); Zahrt, *Mycobacterium tuberculosis signal transduction system required for persistent infections*, Proc. Natl. Acad. Sci. 98 (22), 12706-12711 (2001); and Mundayoor et al.,

Identification of genes involved in the resistance of mycobacteria to killing by macrophages, Ann. N. Y. Acad. Sci. 730, 26-36 (1994).

Bacterial strains and culture conditions

Mycobacterium smegmatis was grown in Middlebrook 7H9 medium (supplier: Difco), supplemented with 10% ADC (Difco), 0.05% Tween-80 and 0.5% glycerol. *E. coli* was cultivated in LB- or TB-broth without any additional ingredients. Cloning, mutagenesis and expression of PknG and other mycobacterial kinases was done as described by Koul et. al. (Serine/threonine kinases, PknG and PknF of *Mycobacterium tuberculosis*: characterisation and localisation. Microbiology, 2001, 147, 2307-23142001).

GST-fusion protein purification

Purification of GST-fusion proteins was done as described previously by Koul et. al. (Serine/threonine kinases, PknG and PknF of *Mycobacterium tuberculosis*: characterisation. and localisation. Microbiology, 2001,147, 2307-.23142001). *E. coli* BL21 cultures containing the respective plasmids were grown overnight in TB-broth. After IPTG induction, the suspensions were incubated for another 16 hours at room temperature. The bacteria were harvested by centrifugation, resuspended in 1x PBS and lysed by sonification. After addition of Triton X-100 (1% final concentration) and subsequent clarifying of the lysates the GST-fusion proteins were purified by addition of GST-sepharose following PBS washes. The proteins were eluted with a buffer containing 50 mM glutathion, 20 mM Tris (pH 8.0), 0.1 M NaCl, 0.1 M Triton X-100 and 1 mM DTT. Thereafter, the eluates were dialysed in 20 mM HEPES (pH 7.5) and 30 % glycerol.

Determination of protein kinase activity

The activity of all protein serine threonine kinases from *Mycobacterium tuberculosis* was determined by addition of myelin basic protein as a substrate in an *in vitro* kinase assay. The buffer conditions were as follows: 20 mM HEPES (pH 7.5), 20 mM $MgCl_2$, and 5 mM $MnCl_2$, for all kinases except PknI, PknJ, and PknL. These protein kinases required lower salt concentrations, namely 1 mM $MgCl_2$, and 1 mM $MnCl_2$. The optimum ATP concentration for each kinase was determined by titration of ATP in a range between 0.0033 µM and 100 µM. The inhibitor studies were performed with ATP concentrations similar to the Michaelis constant (K_m) for ATP. Further, the role of PknG in pathogenesis of mycobacteria in cellular infection model was analysed.

Infection of macrophage cells with recombinant *Mycobacterium smegmatis*

Mycobacterium smegmatis, electroporated with either vector alone or mycobacterial expression vector containing PknG (wild type) or PknG-K181M (Mutant), was cultured for 2 days in Middlebrook 7H9 medium containing 0.05% Tween-80 and 0.5% glycerol. Bacteria were pelleted at 1500 x g for 3 minutes by centrifugation and resuspended by vigorous agitating (Vortex) in Dulbecco's modified Eagle's medium (DMEM, GIBCO-BRL, Gaithersburg, USA)) containing 5 % fetal bovine serum (FBS) for infecting murine macrophage cell line RAW (American Type Culture Collection No. 91B-71). This yielded a bacterial supernatant consisting mostly of single mycobacterial cells as observed by acid fast staining. Under the assumption that an optical density (O.D.) of 0.1 at 650 nm equals to 10^8 CFU/ml (see in this respect Wei et al., *"Identification of a Mycobacterium tuberculosis Gene that enhances survival of M. smegmatis in Macrophages"*, J. Bacteriol. 182, 377-384 (2000)), the O.D. of *Mycobacterium smegmatis* cell suspension was measured and diluted to 5 x 10^6 CFU/ml in DMEM containing 5 % FBS. Viable counts were performed on Middlebrook 7H10 medium.

The RAW cell line was maintained in DMEM medium supplemented with 10 % FBS. The survival assay for recombinant *Mycobacterium smegmatis* was performed as described by Wei et al., cited above. RAW cells were plated in a 24 well tissue culture plate (4 x 10^5 cells/well) and incubated overnight in 5 % CO_2 at 37°C. The inoculum (1 ml) containing 5 x 10^6 recombinant *Mycobacterium smegmatis* was added to achieve muliplicities of infection (moi) of 10. The plate was incubated for 2 hours at 37°C in 5 % CO_2. The infected monolayers were washed twice with warm DMEM and treated with 2 % FCS containing 200 µg of amikacin/ml for 1 hour at 37°C to kill extracellular *M. smegmatis*. The cells were again washed twice with warm DMEM and further incubated in DMEM containing 20 µg of amikacin. This time point was considered 0 hours of infection. The 24 hours infected monolayer was incubated with 20 µg of amikacin/ml to prevent extracellular growth of any bacteria released by premature lysis of infected RAW cells. Cells were washed twice with warm DMEM before lysis was effected by addition of a 0.1 % SDS solution and vigorously pipeting several times to ensure lysis of cells and release of surviving bacteria. The lysates were diluted in 7H9 broth and plated onto 7H10 agar plates and CFU were counted after incubation at 37°C for 4 to 5 days.

Validation of mycobacterial kinase as a mycobacterial virulence gene

Mycobacterium smegmatis was electroporated either with wildtype or mutant kinase (which exerts no kinase activity) or vector control. Mouse macrophage cell line (RAW) was infected with the various recombinant *M. smegmatis* expressing either pknG wild type or PknG K/M mutant or vector alone. After infection, the cells were lysed at different time points and the amount of intracellular bacteria was analysed. As can be seen from Fig. 1, after one hour postinfection the amount of bacteria recovered from macrophages infected with *M. smegmatis* expressing PknG wild type or K/M mutant or vector alone was the same. This shows that the recombinant M. smegmatis strains were internalised with equal efficiency. However, after 24 hour postinfection the amount of *M. smegmatis* transformed with the vector control or the mutant kinase was substantially decreased within macrophages. This shows an efficient clearance or degradation of the the *M.smegmatis* expressing vector alone or PknG K/M mutant by the lysosomal degradation pathway with in the macrophages. But in contrast, after 24 hrs an approximately tenfold increased amount of M.smegmatis survived within the cells expressing wildtype PknG compared to the mutant. This clearly demonstrates that the kinase activity of PknG increases the intracellular survival of *M. smegmatis* within macrophages and as such makes PknG an important virulence factor of mycobacteria. Consequently, the kinase is a promising target for recognising, monitoring, and controlling therapy of various diseases.

Screening for inhibitors of PknG

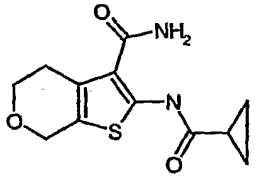
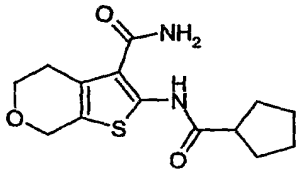
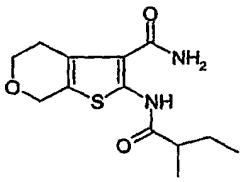
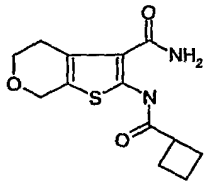
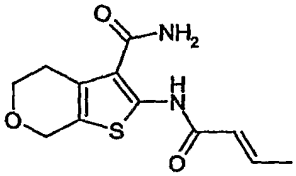
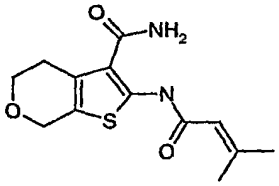
A search was conducted for specific molecules inhibiting the target kinase (PknG) of *Mycobacterium tuberculosis*. In a kinase platform a suitable substrate was identified and an *in vitro* assay was adapted to high throughput screening. The PknG kinase inhibitors were routinely tested under optimised assay conditions: 20 mM Hepes (pH 7.5), 1.8 µM ATP, 1 mM DTT, 10 mM $MnCl_2$. Subsequently, a library comprising 55.000 compounds using the established *in vitro* kinase assay was screened. Table I shows the half-maximal inhibition constant (IC_{50}) values of the compounds 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 1), 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 2), 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3), 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid

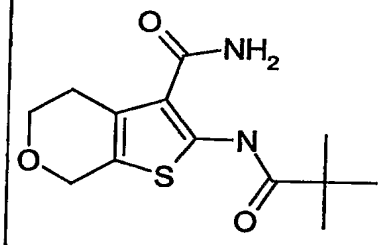
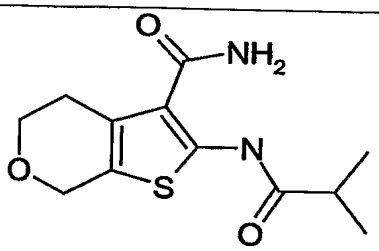
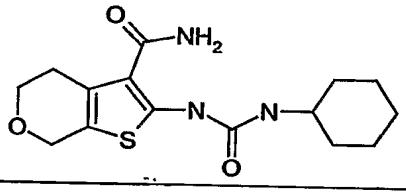
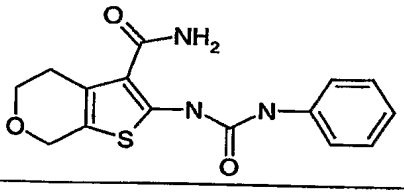
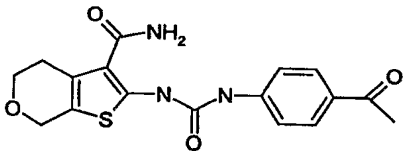
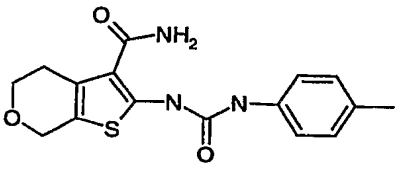
amide (Compound 4), 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6), 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7), 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8), 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10), 2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 18), 2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c] pyran-3-carboxylic acid amide (Compound 19), 2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 20), 2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 21) and 2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 22) for inhibiting mycobacterial PknG .

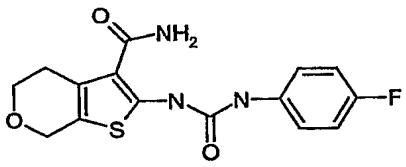
As is evident from Table I, compound 1, compound 18, compound 19, compound 20, compound 21 and compound 22 are the most effective compounds of those tested in inhibiting the activity of protein serine/threonine kinase G of *M. tuberculosis*, compound 1, compound 18, compound 19, compound 20, compound 21 and compound 22 having IC_{50} values between 0,19 μM and 0,71 μM. With compounds 2, 3, 4, 6, 7, 8, and 10 satisfactory results were also obtained, the compounds having IC_{50}-values, between about 2μm and up to about 70 μm.

Table I

Inhibitory effect on mycobacterial protein kinase G (PknG) of selected compounds according the present invention

Compound No.	Structure	Inhibition of PknG (IC_{50}, [µM])
Compound 1: 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**0.49**
Compound 2: 2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**3,42**
Compound 3: 2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**69,2**
Compound 4: 2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**2.17**
Compound 6: 2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c] pyran-3-carboxylic acid amide		**2,26**
Compound 7: 2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**2,64**

Compound No.	Structure	Inhibition of PknG (IC_{50}, [μM])
Compound 8: 2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c] pyran-3-carboxylic acid amide		**57,3**
Compound 10: 2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c] pyran-3-carboxylic acid amide		**20,43**
Compound 18: 2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**0,71**
Compound 19: 2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c] pyran-3-carboxylic acid amide		**0,21**
Compound 20: 2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**0,35**
Compound 21: 2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**0,21**

Compound No.	Structure	Inhibition of PknG (IC_{50}, [µM])
Compound 22: 2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide		**0,19**

Secretion of PknG outside the bacterial cell

In the following it is demonstrated that PknG is secreted outside the cell into the culture supernatant by mycobacterial cells.

1) PknG and ESAT (a secretary protein that acts as a positive control) are cloned in BamH1 site of pYUB 2401. This vector contains the promoter for hsp60. A in-frame fusion with the start of hsp60 and phoA at the C-terminus by cloning into the BamH1 site. The vector is kanamycin resistant. After cloning PknG and ESAT in pYUB2401 they were electroporated in *M. smegmatis* and the colonies were grown on LB plates with 40µg of 5-bromo–4-chloro-3-indoylphosphate (BCIP) and with 20 µg of kanamycin used for screening.

PhoA fusion proteins that are exported beyond cytoplasm are enzymatic ally active and capable of hydrolysing the BCIP, the chromogenic substrate of PhoA to produce the blue colonies.

2) *M. smegmatis* strains containing either
   1) ESAT-PhoA
   2) PknG-PhoA or
   3) PhoA alone

were grown in 7H9 medium with kanamycin to saturation for 5-6 days and then diluted to the final optical density (O.D.) of 0.005 at 600 nm.

3) These cultures were then grown for 40 hours at 37 ^{0}C. The OD_{600} of each strain was measured at the start of the experiment.

4) A 0.5 ml portion of the cell culture was pelleted and resuspended in equal volume 1 M Tris (pH. 8.0).

5) Then 0.1 ml of cells was added to1.0 ml of 2 mM p–nitrophenyl phosphate plus sodium salt in 1 M Tris (pH 8.0).

6) The reaction was incubated in dark at 37 ^{0}C until a yellow reaction product was formed.

7) Next , 0.1 ml of 1 M K_2HP0_4 was added to terminate the reaction.

8) The bacteria were pelleted and the OD_{420} of 1.0 ml of the supernatant was measured.

9) Alkaline phosphatase activity units were determined by the following formula:

$$\frac{1000 \times OD_{420}}{OD_{600} \times 0.1 \text{ ml volume of cells}}$$

The negative control will be the *M. smegmatis* cells alone and PhoA transfected *M. smegmatis*.

The above method is described in Braunstein M, Griffin TJ IV, Kriakov JI, Friedman ST, Grindley ND, Jacobs WR Jr., *„Mycobacterium tuberculosis proteins using a Tn552'phoA in vitro transposition system"*, J Bacteriol. 2000 May;182(10):2732-40.

The result of the above-mentioned experiment shows that PknG is a secretory protein that is secreted outside the mycobacterial cells. Fig. 3 shows the alkaline phosphatase secretions assay for PknG for different PhoA fusion constructs. The secreted PknG can phosphorylate host cell proteins that might be critical in survival of mycobacterium in host cells.

Selectivity panel data:

Table II shows the inhibitory effect of selected compounds according to the present invention on the activity of certain protein kinases. The activity of these protein kinases is depicted as % inhibition in the presence of 10 µM of compound in comparison to DMSO (0% inhibition).

Table II:

Selectivity panel data (% inhibition) of selected compounds according to the present invention

	PDGF-R	c-kit	GSK-3ß	CDK 1	SRPK 1
Compound 1	14	55	38	4	n.a.
Compound 18	20	54	27	50	6
Compound 19	45	73	23	58	68
Compound 20	38	70	14	40	69
Compound 21	25	62	11	29	55
Compound 22	54	64	22	64	56

n.a.: not available

These data show, that compounds according to the present invention, do have a good inhibitory effect on the protein kinase activity of various protein kinases, such as PDGF-R, c-kit, GSK-3ß, CDK 1 and SRPK 1.

The % inhibition values for certain protein kinases shown above were measured according to one of the protocols which are described below. The IC_{50}-value of compounds according to the present invention for inhibiting kinases like for example PDGF-R, c-kit or GSK3-ß were also measured according to the protocols described in the following:

c-Kit-Assay

Reaction Volume:	40 µl
Reaction Time:	60 min
Reaction Temperature:	room temperature
Assay Plate:	96 well U bottom plate (Greiner, 650161)
MultiScreen-PH Plate:	96 well MAPH Filter Plates (Millipore, MAPHNOB50)
Filter Washing Solution:	0.75% H_3PO_4
Szintilation Liquid:	Supermix Liquid Szintillator (PerkinElmer, 1200-439)

Controls:

Negative Control (C-):	100 mM EDTA, no Inhibitor
Positive Control (C+):	no Inhibitor

Reaction Buffer:

20 mM Hepes, pH 7.5
10 mM $MgCl_2$
1 mM DTT
0.01% Tween20

Final Assay Concentrations:

Kinase:	Use kinase conc. yielding 10% ATP turn over.
ATP:	16.9 µM
Adenosine 5'-[γ-^{33}P]triphosphate:	12.5 µCi/ml (Amersham Biosciences, BF1000)
Substrate:	Myelin Basic Protein, 30 µM (Invitrogen, 13228-010)

Pipetting Sequence:

1) Add 10 µl 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate

2) Add 10 µl 4 fold concentrated inhibitor in 4% DMSO in H_2O to each well except to C- and C+ wells
3) Add 10 µl 4% DMSO in H2O to C- and C+ wells
4) Add 10 µl 500 mM EDTA in H_2O to C- wells
5) Add 10 µl 50 µCi/ml Adenosine 5'-[γ-^{33}P]triphosphate in H_2O to each well
6) Add 10 µl 4 fold concentrated kinase in Reaction Buffer to each well
7) Incubate 1hr at room temperature
8) Add 10 µl 50 mM EDTA in H_2O to each well except to C- wells
9) Prepare MAPH plates by adding 200 µl 0.75% H_3PO_4 to each well
10) Exhaust 0.75% H_3PO_4 using Millipore vacuum station
11) Add 60 µl 0.75% H_3PO_4 to each well of MAPH Filter Plate
12) Transfer 30 µl sample per well from Assay Plate to corresponding well of MAPH Filter Plate
13) Incubate 30 min at room temperature
14) Wash each well of MAPH Filter Plates 3x with 200 µl 0.75% H_3PO_4 using Millipore vacuum station
15) Add 20 µl Szintilation Liquid to each well of MAPH Filter Plate
16) Seal MAPH Filter Plate
17) Store MAPH Filter Plate 30 min in darkness
18) Quantify radioactivity

PDGF-R assay:

Reaction Volume:	40 µl
Reaction Time:	60 min
Reaction Temperature:	room temperature
Assay Plate:	96 well U bottom plate (Greiner, 650161)
MultiScreen-PH Plate:	96 well MAPH Filter Plates (Millipore, MAPHNOB50)
Filter Washing Solution:	0.75% H_3PO_4
Szintilation Liquid:	Supermix Liquid Szintillator (PerkinElmer, 1200-439)

Controls:

Negative Control (C-):	100 mM EDTA, no Inhibitor
Positive Control (C+):	no Inhibitor

Reaction Buffer:

20 mM Tris, pH 7.5
10 mM $MgCl_2$
0.4 mM $MnCl_2$
1 mM DTT
0.01% Brij35

Final Assay Concentrations:

Kinase:	Use kinase conc. yielding 10% ATP turn over.
ATP:	16.8 µM
Adenosine 5'-[γ-^{33}P]triphosphate:	12.5 µCi/ml (Amersham Biosciences, BF1000)
Substrate:	Myelin Basic Protein, 20 µM (Invitrogen, 13228-010)

Pipetting Sequence:

1) Add 10 µl 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
2) Add 10 µl 4 fold concentrated inhibitor in 4% DMSO in H_2O to each well except to C- and C+ wells
3) Add 10 µl 4% DMSO in H_2O to C- and C+ wells
4) Add 10 µl 500 mM EDTA in H_2O to C- wells
5) Add 10 µl 50 µCi/ml Adenosine 5'-[γ-^{33}P]triphosphate in H_2O to each well
6) Add 10 µl 4 fold concentrated kinase in Reaction Buffer to each well
7) Incubate 1hr at room temperature
8) Add 10 µl 50 mM EDTA in H_2O to each well except to C- wells
9) Prepare MAPH plates by adding 200 µl 0.75% H_3PO_4 to each well
10) Exhaust 0.75% H_3PO_4 using Millipore vacuum station
11) Add 60 µl 0.75% H_3PO_4 to each well of MAPH Filter Plate
12) Transfer 30 µl sample per well from Assay Plate to corresponding well of MAPH Filter Plate
13) Incubate 30 min at room temperature

14) Wash each well of MAPH Filter Plates 3x with 200 µl 0.75% H_3PO_4 using Millipore vacuum station

15) Add 20 µl Szintilation Liquid to each well of MAPH Filter Plate

16) Seal MAPH Filter Plate

17) Store MAPH Filter Plate 30 min in darkness

18) Quantify radioactivity

GSK-3ß assay

Reaction Volume:	40 µl
Reaction Time:	60 min
Reaction Temperature:	room temperature
Assay Plate:	96 well U bottom plate (Greiner, 650161)
MultiScreen-PH Plate:	96 well MAPH Filter Plates (Millipore, MAPHNOB50)
Filter Washing Solution:	0.75% H_3PO_4
Szintilation Liquid:	Supermix Liquid Szintillator (PerkinElmer, 1200-439)

Controls:

Negative Control (C-):	100 mM EDTA, no Inhibitor
Positive Control (C+):	no Inhibitor

Reaction Buffer:

20 mM Mops, pH 7.0
2 mM $MgCl_2$
1 mM DTT
0.01% Tween20

Final Assay Concentrations:

Kinase:	Use kinase conc. yield. 10% ATP turn over.
ATP:	7.76 µM
Adenosine 5'-[γ-^{33}P]triphosphate:	12.5 µCi/ml (Amersham Biosciences, BF1000)
Substrate:	Phospho-Glycogen Synthase Pept.2, 10 µM (upstate, 12-241)

Pipetting Sequence:

1) Add 10 µl 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
2) Add 10 µl 4 fold concentrated inhibitor in 4% DMSO in H_2O to each well except to C- and C+ wells
3) Add 10 µl 4% DMSO in H_2O to C- and C+ wells
4) Add 10 µl 500 mM EDTA in H_2O to C- wells
5) Add 10 µl 50 µCi/ml Adenosine 5'-[γ-^{33}P]triphosphate in H_2O to each well
6) Add 10 µl 4 fold concentrated kinase in Reaction Buffer to each well
7) Incubate 1hr at room temperature
8) Add 10 µl 50 mM EDTA in H_2O to each well except to C- wells
9) Prepare MAPH plates by adding 200 µl 0.75% H3PO4 to each well
10) Exhaust 0.75% H_3PO_4 using Millipore vacuum station
11) Add 60 µl 0.75% H_3PO_4 to each well of MAPH Filter Plate
12) Transfer 30 µl sample per well from Assay Plate to corresponding well of MAPH Filter Plate
13) Incubate 30 min at room temperature
14) Wash each well of MAPH Filter Plates 3x with 200 µl 0.75% H_3PO_4 using Millipore vacuum station
15) Add 20 µl Szintilation Liquid to each well of MAPH Filter Plate
16) Seal MAPH Filter Plate
17) Store MAPH Filter Plate 30 min in darkness
18) Quantify radioactivity

CDK 1 assay

Reaction Volume:	40 µl
Reaction Time:	60 min
Reaction Temperature:	room temperature
Assay Plate:	96 well U bottom plate (Greiner, 650161)
MultiScreen-PH Plate:	96 well MAPH Filter Plates (Millipore, MAPHNOB50)
Filter Washing Solution:	0.75% H_3PO_4

Szintilation Liquid: Supermix Liquid Szintillator (PerkinElmer, 1200-439)

Controls:

Negative Control (C-): 100 mM EDTA, no Inhibitor
Positive Control (C+): no Inhibitor

Reaction Buffer:

20 mM Mops, pH 7.0
10 mM $MgCl_2$
1 mM DTT
0.01% Brij35

Final Assay Concentrations:

Kinase: Use kinase conc. yielding 10% ATP turn over.
ATP: 27 µM
Adenosine 5'-[γ-^{33}P]triphosphate: 12.5 µCi/ml (Amersham Biosciences, BF1000)
Substrate: PKTPKKAKKL-NH232 µM (Jerini)

Pipetting Sequence:

1) Add 10 µl 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
2) Add 10 µl 4 fold concentrated inhibitor in 4% DMSO in H_2O to each well except to C- and C+ wells
3) Add 10 µl 4% DMSO in H_2O to C- and C+ wells
4) Add 10 µl 500 mM EDTA in H_2O to C- wells
5) Add 10 µl 50 µCi/ml Adenosine 5'-[γ-^{33}P]triphosphate in H_2O to each well
6) Add 10 µl 4 fold concentrated kinase in Reaction Buffer to each well
7) Incubate 1hr at room temperature
8) Add 10 µl 50 mM EDTA in H_2O to each well except to C- wells
9) Prepare MAPH plates by adding 200 µl 0.75% H_3PO_4 to each well

10) Exhaust 0.75% H_3PO_4 using Millipore vacuum station
11) Add 60 µl 0.75% H_3PO_4 to each well of MAPH Filter Plate
12) Transfer 30 µl sample per well from Assay Plate to corresponding well of MAPH Filter Plate
13) Incubate 30 min at room temperature
14) Wash each well of MAPH Filter Plates 3x with 200 µl 0.75% H_3PO_4 using Millipore vacuum station
15) Add 20 µl Szintilation Liquid to each well of MAPH Filter Plate
16) Seal MAPH Filter Plate
17) Store MAPH Filter Plate 30 min in darkness
18) Quantify radioactivity

SRPK 1 assay

Reaction Volume:	40 µl
Reaction Time:	60 min
Reaction Temperature:	room temperature
Assay Plate:	96 well U bottom plate (Greiner, 650161)
MultiScreen-PH Plate:	96 well MAPH Filter Plates (Millipore, MAPHNOB50)
Filter Washing Solution:	0.75% H_3PO_4
Szintilation Liquid:	Supermix Liquid Szintillator (PerkinElmer, 1200-439)

Controls:

Negative Control (C-):	100 mM EDTA, no Inhibitor
Positive Control (C+):	no Inhibitor

Reaction Buffer:

20 mM Hepes, pH 7.5
0.4 mM $MgCl_2$
0.4 mM $MnCl_2$
1 mM DTT
0.01% Tween20

Final Assay Concentrations:

Kinase:	Use kinase conc. yielding 10% ATP turn over.
ATP:	0.56 µM
Adenosine 5'-[γ-^{33}P]triphosphate:	12.5 µCi/ml (Amersham Biosciences, BF1000)
Substrate:	PKC epsilon peptide, 3.1 µM (Biomol, P-155)

Pipetting Sequence:

1) Add 10 µl 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
2) Add 10 µl 4 fold concentrated inhibitor in 4% DMSO in H_2O to each well except to C- and C+ wells
3) Add 10 µl 4% DMSO in H_2O to C- and C+ wells
4) Add 10 µl 500 mM EDTA in H_2O to C- wells
5) Add 10 µl 50 µCi/ml Adenosine 5'-[γ-^{33}P]triphosphate in H_2O to each well
6) Add 10 µl 4 fold concentrated kinase in Reaction Buffer to each well
7) Incubate 1hr at room temperature
8) Add 10 µl 50 mM EDTA in H_2O to each well except to C- wells
9) Prepare MAPH plates by adding 200 µl 0.75% H_3PO_4 to each well
10) Exhaust 0.75% H_3PO_4 using Millipore vacuum station
11) Add 60 µl 0.75% H_3PO_4 to each well of MAPH Filter Plate
12) Transfer 30 µl sample per well from Assay Plate to corresponding well of MAPH Filter Plate
13) Incubate 30 min at room temperature
14) Wash each well of MAPH Filter Plates 3x with 200 µl 0.75% H_3PO_4 using Millipore vacuum station
15) Add 20 µl Szintilation Liquid to each well of MAPH Filter Plate
16) Seal MAPH Filter Plate
17) Store MAPH Filter Plate 30 min in darkness
18) Quantify radioactivity

According to one of the protocols described above, for 2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (compound 1) the following IC_{50}-values for inhibiting the protein kinases c-kit, GSK-3ß And PDGF-R were obtained:

Inhibition of c-kit :	5.4µM (IC_{50}),
Inhibition of GSK-3ß:	27 µM (IC_{50}),
Inhibition of PDGF-R:	90 µM (IC_{50}).

General kinase assay:

Table III shows all currently known protein kinases. The inhibitory effect of compounds according to the present invention on the activity of these protein kinases may be measured according to the following protocol:

Reaction Volume:	40 µl
Reaction Time:	60 min
Reaction Temperature:	room temperature
Assay Plate:	96 well U bottom plate (Greiner, 650161)
MultiScreen-PH Plate:	96 well MAPH Filter Plates (Millipore, MAPHNOB50)
Filter Washing Solution:	0.75% H_3PO_4
Szintilation Liquid:	Supermix Liquid Szintillator (PerkinElmer, 1200-439)

Controls:

Negative Control (C-):	100 mM EDTA, no Inhibitor
Positive Control (C+):	no Inhibitor

Reaction Buffer:

20 mM Tris, pH 7.5
10 mM $MgCl_2$
1 mM DTT

Final Assay Concentrations:

Kinase:	Use kinase conc. yielding 10% ATP turn over.
ATP:	1 µM
Adenosine 5'-[γ-^{33}P]triphosphate:	12.5 µCi/ml (Amersham Biosciences, BF1000)
Substrate:	Myelin Basic Protein, 10 µM (Invitrogen, 13228-010)

Pipetting Sequence:

1) Add 10 µl 4 fold concentrated Substrate + 4 fold concentrated ATP in 3 fold concentrated Reaction Buffer to each well of Assay Plate
2) Add 10 µl 4 fold concentrated inhibitor in 4% DMSO in H_2O to each well except to C- and C+ wells
3) Add 10 µl 4% DMSO in H_2O to C- and C+ wells
4) Add 10 µl 500 mM EDTA in H_2O to C- wells
5) Add 10 µl 50 µCi/ml Adenosine 5'-[γ-^{33}P]triphosphate in H_2O to each well
6) Add 10 µl 4 fold concentrated kinase in Reaction Buffer to each well
7) Incubate 1hr at room temperature
8) Add 10 µl 50 mM EDTA in H_2O to each well except to C- wells
9) Prepare MAPH plates by adding 200 µl 0.75% H_3PO_4 to each well
10) Exhaust 0.75% H_3PO_4 using Millipore vacuum station
11) Add 60 µl 0.75% H_3PO_4 to each well of MAPH Filter Plate
12) Transfer 30 µl sample per well from Assay Plate to corresponding well of MAPH Filter Plate
13) Incubate 30 min at room temperature
14) Wash each well of MAPH Filter Plates 3x with 200 µl 0.75% H_3PO_4 using Millipore vacuum station
15) Add 20 µl Szintilation Liquid to each well of MAPH Filter Plate
16) Seal MAPH Filter Plate
17) Store MAPH Filter Plate 30 min in darkness
18) Quantify radioactivity

Table III: List of protein kinases

No.	Accession Number	Gene
1	NM_001105	ACVR1 (activin A receptor, type I)
2	NM_004302 NM_020327 NM_020328	ACVR1B (activin A receptor, type IB)
3	NM_145259	ACVR1C, ALK7
4	NM_001616	ACVR2, activin A receptor, type II
5	NM_001106	ACVR2B, activin A receptor, type IIB
6	NM_000020	ACVRL1 (activin A receptor type II-like 1)
7	NM_004612	TGFBR1 (transforming growth factor, beta receptor I (activin A receptor type II-like kinase, 53kD))
8	NM_003242	TGFBR2 (transforming growth factor, beta receptor II)
9	NM_004329	BMPR1A (bone morphogenetic protein receptor, type IA)
10	NM_001203	BMPR1B (bone morphogenetic protein receptor, type IB)
11	NM_001204	BMPR2 (bone morphogenetic protein receptor, type II (serine/threonine kinase))
12	NM_006251	PRKAA1 (protein kinase, AMP-activated, alpha 1 catalytic subunit)
13	NM_006252	PRKAA2 (protein kinase, AMP-activated, alpha 2 catalytic subunit)
14	NM_002929	GRK1; rhodopsin kinase
15	NM_001619	GRK2

No.	Accession Number				Gene
16	NM_005160				GRK3
17	NM_005307	NM_182982			GRK4
18	NM_005308				GRK5
19	NM_002082				GRK6
20	NM_139209				GRK7 (G protein-coupled receptor kinase 7)
21	NM_017572				MKNK2, GPRK7
22	NM_001654				ARAF1 (v-raf murine sarcoma 3611 viral oncogene homolog 1)
23	NM_004333				BRAF (v-raf murine sarcoma viral oncogene homolog B1)
24	NM_002880				RAF1 (v-raf-1 murine leukemia viral oncogene homolog 1)
25	NM_021574	NM_004327	no kinase domain	has STK activity	BCR1
26	NM_003656				CAMK1 (calcium/calmodulin-dependent protein kinase I)
27	NM_015981	NM_171825			CAMK2A (calcium/calmodulin-dependent protein kinase (CaM kinase) II alpha)
28	NM_001220				CAMK2B (calcium/calmodulin-dependent protein kinase (CaM kinase) II beta)
29	NM_001221				CAMK2D (calcium/calmodulin-dependent protein kinase (CaM kinase) II delta)
30	NM_020439				CAMK1G (calcium/calmodulin-dependent protein kinase IG)

No.	Accession Number						Gene
31	NM_001222	NM_172169	NM_172170	NM_172171	NM_172172	NM_172173	CAMK2G (calcium/calmodulin-dependent protein kinase (CaM kinase) II gamma)
32	NM_001744						CAMK4 (calcium/calmodulin-dependent protein kinase IV)
33	NM_001786	NM_033379					CDC2 (cell division cycle 2)
34	NM_001798	NM_052827					CDK2 (cyclin-dependent kinase 2)
35	NM_001258						CDK3 (cyclin-dependent kinase 3)
36	NM_000075	NM_032913					CDK4 (cyclin-dependent kinase 4)
37	NM_004935						CDK5 (cyclin-dependent kinase 5)
38	NM_001259						CDK6 (cyclin-dependent kinase 6)
39	NM_001799						CDK7 (cyclin-dependent kinase 7)
40	NM_001260						CDK8 (cyclin-dependent kinase 8)
41	NM_001261						CDK9 (cyclin-dependent kinase 9 (CDC2-related kinase))
42	NM_003674						CDK10 (cyclin-dependent kinase (CDC2-like) 10)
43	NM_015076						CDK11, DPK
44	NM_004196						CDKL1 (cyclin-dependent kinase-like 1); KKIALRE
45	NM_003948						CDKL2 (cyclin-dependent kinase-like 2); KKIAMRE
46	NM_016508						CDKL3 (cyclin-dependent kinase-like 3); NKIAMRE
47	XM_293029	AX166534	cds: 1-1083				CDKL4, similar to cyclin-dependent kinase-like 1
48	NM_033489	8 transcripts					CDC2L1 (cell division cycle 2-like 1); PITSLRE B
49	NM_024011	NM_033536	9 transcripts				CDC2L1 (cell division cycle 2-like 1); PITSLRE A
50	NM_003718	NM_031267					CDC2L5 (cell division cycle 2-like 5)

No.	Accession Number			Gene
51	NM_006201	NM_033018	NM_033019	PCTK1 (PCTAIRE protein kinase 1)
52	NM_002595			PCTK2 (PCTAIRE protein kinase 2)
53	NM_002596			PCTK3 (PCTAIRE protein kinase 3)
54	NM_012395			PFTK1 (PFTAIRE protein kinase 1)
55	NM_001278			IKK-alpha; CHUK
56	NM_001556	XM_032491		IKK-beta; IKK2
57	NM_001892			CSNK1A1 (casein kinase 1, alpha 1)
58	NM_001893			CSNK1D (casein kinase 1, delta)
59	NM_001894			CSNK1E (casein kinase 1, epsilon)
60	NM_004384			CSNK1G3 (casein kinase 1, gamma 3)
61	NM_001319			CSNK1G2 (casein kinase 1, gamma 2)
62	NM_001895			CSNK2A1 (casein kinase 2, alpha 1)
63	NM_001896			CSNK2A2 (casein kinase 2, alpha prime)
64	NM_022048			CSNK1G1 (casein kinase 1, gamma 1)
65	NM_004071			CLK1 (CDC-like kinase 1)
66	NM_003993	NM_001291		CLK2 (CDC-like kinase 2)
67	NM_003992	NM_001292		CLK3 (CDC-like kinase 3)
68	NM_020666			CLK4 (CDC-like kinase 4)
69	NM_004938			DAPK1 (death-associated protein kinase 1)
70	NM_014326			DAPK2 (death-associated protein kinase 2)
71	NM_001348			DAPK3 (death-associated protein kinase 3)
72	NM_004954	NM_017490		EMK1 (ELKL motif kinase)
73	NM_002746			MAPK3; ERK1

No.	Accession Number					Gene
74	NM_002745					MAPK1, ERK2
75	NM_002748					MAPK6; ERK3
76	NM_002747					MAPK4; ERK3-related
77	NM_002749					MAPK7; ERK5
78	NM_001315	NM_139012	NM_139013	NM_139014		MAPK14; CSBP1
79	NM_002751	NM_138993				MAPK11; p38beta
80	NM_002969					MAPK12; ERK6, p38g
81	NM_002754					MAPK13; p38delta
82	AY065978	AX056411	cds 30-1664	NM_139021		ERK8
83	NM_002750					MAPK8; JNK1
84	NM_002752					MAPK9; JNK2
85	NM_002753					MAPK10; JNK3
86	NM_006712	NM_033015	no kinase domain		has STK activity	FASTK (Fas-activated protein kinase)
87	NM_004579					MAP4K2; GCK
88	NM_019884					GSK3A (glycogen synthase kinase 3 alpha)
89	NM_002093					GSK3B (glycogen synthase kinase 3 beta)
90	NM_002576					PAK1
91	NM_002577					PAK2
92	NM_002578					PAK3
93	NM_005884					PAK4
94	NM_020341					PAK5 (PAK7)
95	NM_020168					PAK6

No.	Accession Number		Gene
96	NM_007181		MAP4K1; HPK1
97	NM_004517		ILK (integrin-linked kinase)
98	NM_001569		IRAK1 (interleukin-1 receptor-associated kinase 1)
99	NM_001570		IRAK2 (interleukin-1 receptor-associated kinase 2)
100	NM_007199		IRAK-M
101	NM_016123		IRAK4
102	NM_006575		MAP4K5
103	NM_002314	NM_016735	LIMK1 (LIM domain kinase 1)
104	NM_005569	NM_016733	LIMK2 (LIM domain kinase 2)
105	NM_000455		STK11; LKB1
106	NM_005906		MAK (male germ cell-associated kinase)
107	NM_002755		MAP2K1; MEK1
108	NM_030662		MAP2K2; MEK2
109	NM_002756		MAP2K3; MEK3
110	NM_003010		MAP2K4; MEK4
111	NM_002757		MAP2K5; MEK5
112	NM_002758	NM_031988	MAP2K6; MEK6
113	NM_005043		MAP2K7; MKK7
114	XM_042066	AF042838	MAP3K1; MEKK1
115	NM_006609		MAP3K2; MEKK2
116	NM_002401		MAP3K3; MEKK3
117	NM_005922	NM_006724	MAP3K4; MEKK4
118	NM_005923		MAP3K5; ASK1

No.	Accession Number			Gene
119	NM_004672			MAP3K6
120	NM_003188			MAP3K7; TAK1
121	NM_005204			MAP3K8; Tpl-2
122	XM_027237	AF251442		MAP3K9; MLK1
123	NM_002446			MAP3K10; MST; MLK2
124	NM_002419			MAP3K11; MLK3
125	NM_006301			MAP3K12; DLK
126	NM_004721			MAP3K13; LZK
127	NM_003954			MAP3K14; NIK
128	AX282911	cds: 1 - 4080	XM_372199	MAP3K7, similar to MAP/ERK kinase kinase 5; apoptosis signal regulating kinase
129	AX504239	cds: 1 - 2208	AK122935	MAP3K8
130	NM_015112			MAST205
131	NM_005965			MYLK (myosin, light polypeptide kinase)
132	NM_033118			MYLK2 (myosin light chain kinase 2)
133	NM_005372			MOS (v-mos Moloney murine sarcoma viral oncogene homolog)
134	NM_006282			STK4; MST1
135	NM_006281			STK3; MST2
136	NM_003576			STK24; MST3
137	NM_012224			NEK1 (NIMA (never in mitosis gene a)-related kinase 1)
138	NM_002497			NEK2 (NIMA (never in mitosis gene a)-related kinase 2)
139	NM_002498	Z29067		NEK3 (NIMA (never in mitosis gene a)-related kinase 3)

No.	Accession Number			Gene
140	AX394707	XM_292160		NEK5
141	NM_014397			NEK6 (NIMA (never in mitosis gene a)-related kinase 6)
142	NM_133494	AR130839 (ext 3 non-coding region)	cds: 297 - 1205	NEK7
143	NM_178170			NEK8, NEK12A
144	NM_033116	AR100127		NEK9
145	AX250157	cds:1-2561	NM_152534	NEK10
146	NM_024800	NM_145910		NEK11
147	NM_003157			STK2
148	NM_005406			ROCK1 (Rho-associated, coiled-coil containing protein kinase 1); p160ROCK
149	NM_004850			ROCK2 (Rho-associated, coiled-coil containing protein kinase 2)
150	NM_007271			STK38; NDR
151	NM_015000			STK38L, NDR2
152	NM_004409			DMPK1 (dystrophia myotonica-protein kinase)
153	XM_290516			DMPK2, HSMDPKIN
154	NM_003607			MRCKalpha (PK428)
155	NM_007174	AX166510	AB023166 (C-Term. Longer)	Citron
156	NM_002613			PDPK1 (3-phosphoinositide dependent protein kinase-1)
157	NM_006213			PHKG1 (phosphorylase kinase, gamma 1)
158	NM_000294			PHKG2 (phosphorylase kinase, gamma 2)
159	NM_002648			PIM1
160	NM_006875			PIM2

No.	Accession Number		Gene
161	AR208686		PIM3
162	NM_014791		KIAA0175
163	NM_002730		PRKACA (protein kinase, cAMP-dependent, alpha)
164	NM_002731		PRKACB (protein kinase, cAMP-dependent, beta)
165	NM_002732		PRKACG (protein kinase, cAMP-dependent, gamma)
166	NM_002742		PRKCM (protein kinase C, mu)
167	NM_002737		PRKCA (protein kinase C, alpha)
168	NM_002738	X07109	PRKCB1 (protein kinase C, beta 1)
169	NM_006254		PRKCD (protein kinase C, delta)
170	NM_005400		PRKCE (protein kinase C, epsilon)
171	NM_002739		PRKCG (protein kinase C, gamma)
172	NM_006255		PRKCH (protein kinase C, eta)
173	NM_002740		PRKCI (protein kinase C, iota)
174	NM_006257		PRKCQ (protein kinase C, theta)
175	NM_002744		PRKCZ (protein kinase C, zeta)
176	NM_002741		PRKCL1 (protein kinase C-like 1)
177	NM_006256		PRKCL2 (protein kinase C-like 2)
178	NM_006258		PRKG1 (protein kinase, cGMP-dependent, type I)
179	NM_006259		PRKG2 (protein kinase, cGMP-dependent, type II); cGKII
180	NM_002759		PRKR (protein kinase, interferon-inducible double stranded RNA dependent)
181	NM_006852		TLK2 (tousled-like kinase 2)

No.	Accession Number		Gene
182	NM_012290		TLK1 (tousled-like kinase 1)
183	NM_005044		PRKX (protein kinase, X-linked)
184	NM_005030		PLK (polo-like kinase)
185	NM_004073		CNK (cytokine-inducible kinase)
186	NM_003913	NM_176800	PRPF4B
187	NM_006742		PSKH1 (protein serine kinase H1)
188	NM_005163		AKT1 (v-akt murine thymoma viral oncogene homolog 1)
189	NM_001626		AKT2 (v-akt murine thymoma viral oncogene homolog 2)
190	NM_005465		AKT3 (v-akt murine thymoma viral oncogene homolog 3 (protein kinase B, gamma))
191	NM_014264		STK18; Sak
192	NM_005627		SGK (serum/glucocorticoid regulated kinase)
193	NM_002376		MARK3 (MAP/microtubule affinity-regulating kinase 3)
194	NM_006374		STK25; YSK1
195	NM_003137		SRPK1 (SFRS protein kinase 1)
196	NM_182692	NM_182691	SRPK2 (SFRS protein kinase 2)
197	NM_003319		Titin
198	NM_003318		TTK protein kinase
199	NM_003384		VRK1 (vaccinia related kinase 1)
200	NM_006296		VRK2 (vaccinia related kinase 2)
201	NM_003390		WEE1
202	NM_018650	AB040910	MARK1 (MAP/microtubule affinity-regulating kinase 1)
203	NM_003160		STK13; (aurora/IPL1-like), AIE2, aurora kinase C

No.	Accession Number			Gene
204	NM_004759	NM_032960		MAPKAPK2
205	NM_004635			MAPKAPK3
206	NM_003668			MAPKAPK5
207	NM_005734			HIPK3 (homeodomain interacting protein kinase 3), DYRK6
208	NM_003503			CDC7L1 (CDC7 cell division cycle 7-like 1)
209	NM_016231			NLK
210	NM_003565			ULK1 (unc-51-like kinase 1)
211	NM_014683			ULK2 (unc-51-like kinase 2)
212	AX056454	AL117482		DKFZP434C131 protein, ULK3
213	NM_017886	AX428076	cds:1-1743	hypothetical protein FLJ20574, ULK4
214	NM_053006			STK22B; TSSK2
215	NM_003684	NM_198973		MKNK1 (MAP kinase-interacting serine/threonine kinase 1); MNK1
216	NM_003804			RIPK1 (receptor (TNFRSF)-interacting serine-threonine kinase 1); RIP
217	NM_003821			RIPK2 (receptor-interacting serine-threonine kinase 2); RICK
218	NM_006871			RIPK3 (receptor-interacting serine-threonine kinase 3); RIP3
219	NM_003600			STK6; BTAK, AIK
220	NM_004217			STK12; IPL1, aurora kinase B

No.	Accession Number			Gene
221	NM_006549			CAMKK2 (calcium/calmodulin-dependent protein kinase kinase 2, beta)
222	NM_017719			SNRK (SNF-1 related kinase)
223	NM_001433	AF059198		ERN1 (ER to nucleus signalling 1)
224	NM_004336			BUB1 (BUB1 budding uninhibited by benzimidazoles 1 homolog)
225	NM_001211			BUB1B (BUB1 budding uninhibited by benzimidazoles 1 homolog beta)
226	NM_006622			SNK (serum-inducible kinase)
227	NM_001274			CHEK1 (CHK1 checkpoint homolog)
228	NM_003957	AJ006701	AF020089	STK29; PEN11B
229	NM_013233			STK39; SPAK
230	NM_003691			STK16; PKL12
231	XM_290796	AY049015	AK024376	TAO1/KIAA1361
232	NM_003159			STK9
233	NM_014586			HUNK (hormonally upregulated Neu-associated kinase)
234	NM_004834	NM_145686	NM_145687	MAP4K4; NIK; HGK
235	NM_002953			RPS6KA1 = ribosomal protein S6 kinase, 90kD, polypeptide 1
236	NM_021135			RPS6KA2 (ribosomal protein S6 kinase, 90kD, polypeptide 2); RSK3
237	NM_003161			RPS6KB1 (ribosomal protein S6 kinase, 70kD, polypeptide 1)

No.	Accession Number	Gene
238	NM_004586	RPS6KA3 = ribosomal protein S6 kinase, 90kD, polypeptide 3; RSK2
239	NM_004755	RPS6KA5 (ribosomal protein S6 kinase, 90kD, polypeptide 5); MSK1
240	NM_003942	RPS6KA4 (ribosomal protein S6 kinase, 90kD, polypeptide 4); MSK2
241	NM_003952	RPS6KB2 (ribosomal protein S6 kinase, 70kD, polypeptide 2)
242	NM_004760	STK17A; DRAK1
243	NM_014413	HRI (heme-regulated initiation factor 2-alpha kinase)
244	NM_007194	CHEK2 (CHK2 checkpoint homolog)
245	NM_012119	CCRK (cell cycle related kinase)
246	NM_014370	STK23; MSSK1
247	NM_005990	STK10; LOK
248	NM_004836	EIF2AK3 (eukaryotic translation initiation factor 2-alpha kinase 3)
249	NM_003618	MAP4K3; GLK
250	NM_014720	SLK (SNF1 sucrose nonfermenting like kinase)
251	NM_014602	PIK3R4 (phosphoinositide-3-kinase, regulatory subunit 4, p150)
252	NM_006285	TESK1 (testis-specific kinase 1)
253	NM_021643	GS3955 protein
254	NM_004203	PKMYT1

No.	Accession Number					Gene
255	NM_015148					PASK (PAS domain containing serine/threonine kinase)
256	NM_014002					IKKE (IKK-related kinase epsilon; inducible IkappaB kinase)
257	NM_007118					TRIO (triple functional domain (PTPRF interacting))
258	NM_001396					DYRK1A (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1A)
259	NM_004714	NM_006483	NM_006484			DYRK1B (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 1B)
260	NM_003583	NM_006482				DYRK2 (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2)
261	NM_003582					DYRK3 (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3)
262	NM_003845	AX166542 (longer at N-Term, C-Term differnt)				DYRK4 (dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 4)
263	NM_031417					MARKL1 (MAP/microtubule affinity-regulating kinase like 1)
264	NM_014840					KIAA0537 gene product
265	XM_039796	8 splice variants		AF172264 - AF172271		TNIK (Traf2 and NCK interacting kinase)
266	XM_038150	AB011133				MAST3, KIAA0561 protein
267	XM_291141	AX166512	cds: 1-7572	AX766336	cds: 347-7636	MAST4, KIAA0303 protein
268	NM_015375	AB007941				DustyPK
269	NM_002760					PRKY (protein kinase, Y-linked)

No.	Accession Number			Gene
270	NM_003688			CASK (calcium/calmodulin-dependent serine protein kinase (MAGUK family))
271	NM_004734			DCAMKL1 (doublecortin and CaM kinase-like 1)
272	NM_152619	AX056380	cds: 7 - 2082	hypothetical protein MGC45428, DCAMKL2
273	AX504237	XM_047355 with ATG	XM_047355 with ATG	DCAMKL3, KIAA1765 protein
274	NM_004226			STK17B; DRAK2
275	NM_005813			PRKCN (protein kinase C, nu)
276	NM_005255			GAK (cyclin G associated kinase)
277	NM_032294			hypothetical protein DKFZp761M0423
278	NM_014226			RAGE1 (renal tumor antigen)
279	NM_006035			CDC42BPB (CDC42 binding protein kinase beta (DMPK-like))
280	NM_007170			TESK2 (testis-specific kinase 2)
281	NM_152696			Nbak2, KIAA0630 protein
282	NM_016151			PSK
283	NM_173354	AX224729	cds: 112-2463	SNF1LK, SIK
284	AB023190			SAST (syntrophin associated serine/threonine kinase)
285	NM_022740			HIPK2 (homeodomain interacting protein kinase 2)
286	AX236110	cds:63-5012	AB037759	GCN2, eIF2alpha kinase
287	NM_013355			PKNbeta
288	NM_198465	AX504249	cds: 294-5039	NRK/ZC4 (NIK-related kinase)
289	NM_013257			SGKL (serum/glucocorticoid regulated kinase-like)

No.	Accession Number			Gene
290	NM_016276			SGK2 (serum/glucocorticoid regulated kinase 2)
291	NM_012424			RPS6KC1 (ribosomal protein S6 kinase, 52kD, polypeptide 1)
292	NM_014496			RPS6KA6 (ribosomal protein S6 kinase, 90kD, polypeptide 6); RSK4
293	NM_013254			TBK1 (TANK-binding kinase 1)
294	NM_016281			JIK
295	NM_016440			VRK3 for vaccinia related kinase 3
296	NM_015716			MINK (Misshapen/NIK-related kinase)
297	AX166520	cds: 1-1032		similar to Ca2+/Calmodulin-dependent protein kinase I, CAMK1b
298	NM_006410			HTATIP2 (HIV-1 Tat interactive protein 2, 30 kD)
299	NM_016542			MST4
300	NM_016653			ZAK (sterile-alpha motif and leucine zipper containing kinase AZK)
301	NM_173575			PKE, YANK3
302	NM_018979			PRKWNK1 (protein kinase, lysine deficient 1); WNK1
303	NM_006648	AX540385		PRKWNK2 (protein kinase, lysine deficient 2)
304	NM_020922	AJ409088	AX207405 splice variant?	PRKWNK3 (protein kinase, lysine deficient 3)
305	NM_032387			PRKWNK4 (protein kinase, lysine deficient 4)
306	NM_018492			TOPK (T-LAK cell-originated protein kinase)
307	AL359916 (longer at 5')	NM_080836		STK35, CLIK1

No.	Accession Number				Gene
308	NM_020680				NTKL (N-terminal kinase-like)
309	NM_032844				MASTL, hypothetical protein FLJ14813
310	NM_020397	NM_153498			CKLiK, CamKI-like protein kinase
311	AX224725	cds:1-2379	AB037781 (longer 3')	NM_017988	SCYL2
312	NM_153335	AF308302	AF308302		STLK5, LYK5
313	NM_174944	AX056447	cds: 372-1247		TSSK4
314	NM_052841				STK22C; TSSK3
315	XM_166453	AB058758			TTBK1
316	AR004796	U43586	XM_290793		KSR1 (kinase suppressor of ras)
317	NM_032037				SSTK
318	NM_016457				PKD2 (polycystic kidney disease 2)
319	NM_025195				C8FW, Trb1
320	NM_033266				ERN2 (ER to nucleus signalling 2)
321	NM_020423				PACE-1
322	NM_033550	AB017505			PRPK
323	NM_018401				serine/thronine kinase HSA250839, YANK2
324	NM_020639				ANKRD3 (ankyrin repeat domain 3); DIK
325	NM_015690				STK36
326	NM_014572				LATS2 (LATS, large tumor suppressor, homolog 2)
327	AX056397	cds:7-6861	AB037718		SPEG, KIAA1297 protein
328	AX504253	cds: 1-1704			Wee1B
329	AX766335	cds:1-4110	AB023216	NM_025164 shorter	QSK, KIAA0999 protein

No.	Accession Number			Gene
330	NM_007064			TRAD
331	NM_004690			LATS1 (LATS, large tumor suppressor, homolog 1)
332	NM_014911			AAK1
333	NM_014920	NM_016513	NM_173041	ICK, MAK-related kinase
334	NM_198892	NM_017593		BMP2K, BIKE
335	NM_033126			PSKH2
336	NM_031464			hypothetical protein MGC11287 similar to ribosomal protein S6 kinase
337	NM_032409			PINK1 (PTEN induced putative protein kinase 1)
338	NM_013392			NRBP (nuclear receptor binding protein
339	NM_016507			CrkRS
340	NM_005109			OSR1 (oxidative-stress responsive 1)
341	NM_139158			ALS2CR7
342	NM_032028	AY028964		STK22D, TSSK1
343	NM_017771			PXK (PX domain-containing protein kinase), Slob
344	NM_018571			ALS2CR2 (amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2), STLK6
345	NM_031965			GSG2, haspin
346	NM_015191			SIK2, QIK
347	AX039412	cds:60 - 7850	AB046859 N-Term missing	KIAA1639, Obscn

No.	Accession Number				Gene
348	AX207388	cds: 404 - 1591	NM_145001		YANK1
349	AX394712	cds:282-1448	XM_373109		similar to MLCK, hypothetical protein LOC340156
350	NM_178510	AX207411	cds: 54 - 2348		ANKK1
351	NM_021158				C20orf97 (chromosome 20 open reading frame 97), Trb3
352	NM_152649	AX224735	cds: 465 - 1880		MLKL, hypothetical protein FLJ34389
353	AX250159	cds: 1 - 3735	XM_291277		SgK223, DKFZp761P0423
354	XM_370878	AX250160			KIAA2002
355	NM_024652	AX250161	cds: 1 - 6044		LRRK1
356	NM_033115	AX250162	cds: 358-3039		TBCK, hypothetical portein MGC16169
357	AX250163	cds: 1-1682			SgK424, similar to testis expressed gene 14 (LOC126392)
358	NM_031272	NM_198393	AX250165	cds: 123 - 4409	TEX14 (testis expressed sequence 14)
359	NM_024046				hypothetical protein MGC8407, VACAMKL
360	NM_014916				LMTK2, KIAA1079 protein, LMR2, KPI-2
361	NM_017433				MYO3A
362	NM_138995				MYO3B
363	NM_030952				SNARK
364	NM_030906	AJ303380			STK33

No.	Accession Number				Gene
365	NM_182493				similar to myosin light chain kinase (MLCK)
366	NM_032430	AB058714			BRSK1, KIAA1811
367	XM_370948	AX166553	cds: 1 - 1275		SBK, similar to SH3-binding kinase (LOC388228)
368	NM_032017				SINK-homologous serine/threonine kinase, MGC4796
369	NM_020547				AMHR2 (anti-Mullerian hormone receptor, type II)
370	NM_031414	NM_032944			STK31
371	NM_032237				hypothetical protein FLJ23356
372	NM_021133				RNASEL (ribonuclease L (2',5'-oligoisoadenylate synthetase-dependent))
373	AX166516	XM_372749			similar to protein kinase Bsk146
374	NM_153361				NIM1, MGC42105, similar to serine/threonine kinase (KIN1/SNF1/Nim1 subfamily)
375	NM_145203				casein kinase 1 alpha S-like, CKIa2
376	NM_173500	AX262516	cds: 317 - 4051		TTBK2
377	NM_144685				HIPK4
378	NM_175866	NM_144624	AX262519		KIS
379	AX166547	cds: 1 - 2835	NM_173598		KSR2
380	AX056416	cds: 28 - 1389	AR448352	cds: cds:282- 1529	NRBP2
381	AX540378	cds: 192- 1541	NM_144610	AL137662	SgK494, hypothetical protein FLJ25006
382	NM_152835				CLIK1L

No.	Accession Number			Gene
383	AX540373	cds: 195-2073	XM_376950	SgK071, similar to MGC43306 protein (LOC401568)
384	AX056460	cds: 1 - 1623	XM_038576	SgK493, hypothetical protein BC007901 (LOC91461)
385	NM_005157	NM_007313		ABL1
386	NM_005158	NM_007314		ABL2, ARG
387	NM_005781			ACK1
388	NM_000061			BTK
389	NM_005246			FER
390	NM_002005			FES
391	NM_002031			FRK (fyn-related kinase)
392	NM_002037	NM_153047	NM_153048	FYN
393	NM_002110			HCK
394	NM_005248			FGR
395	NM_005356			LCK
396	NM_002344			LTK
397	NM_002350			LYN
398	NM_004383			CSK
399	NM_005546			ITK
400	NM_005417	NM_198291		SRC
401	NM_003215			TEC
402	NM_005433			YES
403	NM_003328			TXK
404	NM_080823	AL121829 genomic clone		SRMS

No.	Accession Number			Gene
405	NM_001715			BLK
406	NM_001721			BMX
407	NM_005975			PTK6
408	NM_002821			PTK7
409	NM_002822			PTK9
410	NM_007284			PTK9L
411	NM_000222			KIT
412	NM_005211			CSF1R
413	NM_005232			EphA1
414	NM_004431			EphA2
415	NM_005233	NM_182644		EphA3
416	NM_004438			EphA4
417	NM_004439	NM_182472		EphA5
418	AX250164	cds: 280-3390	XM_114973	EphA6
419	NM_004440			EphA7
420	NM_020526			EphA8
421	AX166562	cds: 74 - 3100		EphA10
422	NM_004441			EphB1
423	NM_004442	NM_017449	AF025304	EphB2
424	NM_004443			EphB3
425	NM_004444			EphB4
426	NM_004445			EphB6

No.	Accession Number			Gene
427	NM_000604	9 transcripts	NM_023109	FGFR1
428	NM_000141	13 transcripts	NM_023028	FGFR2
429	NM_000142	NM_022965		FGFR3
430	NM_002011	NM_022963		FGFR4
431	NM_002253			KDR
432	NM_002019			FLT1
433	NM_004119			FLT3
434	NM_002020			FLT4
435	NM_005228			EGFR
436	NM_004448			HER2
437	NM_001982			HER3
438	NM_005235			HER4
439	NM_002378			MATK
440	NM_000875			IGF1R
441	NM_000208			INSR
442	NM_014215			INSRR
443	NM_002227			JAK1
444	NM_004972			JAK2
445	NM_000215			JAK3
446	NM_003331			TYK2
447	NM_006343			MER
448	NM_021913	NM_001699		AXL

No.	Accession Number				Gene
449	NM_006293				TYRO3
450	NM_000245				MET
451	NM_002447				MST1R, RON
452	NM_002958				RYK
453	NM_006206				PDGFRalpha
454	NM_002609				PDGFRbeta
455	NM_020630	NM_020629	NM_000323	NM_020975	RET
456	NM_005012				ROR1
457	NM_004560				ROR2
458	NM_002944				ROS1
459	NM_005607	NM_153831			PTK2, FAK
460	NM_004103	NM_173174	NM_173175	NM_173176	PTK2B, PYK2
461	NM_003177				SYK
462	NM_001079				ZAP70
463	NM_005424				TIE1
464	NM_000459				TEK, TIE2
465	NM_005592				MUSK
466	NM_002529				NTRK1
467	NM_006180				NTRK2
468	NM_002530				NTRK3
469	NM_013994	NM_001954	NM_013993		DDR1
470	NM_006182				DDR2
471	NM_004920				AATK/LMR1

No.	Accession Number				Gene
472	XM_055866				LMTK3
473	NM_003985				TNK1
474	L08961				HUMSPRMTK
475	NM_004304				ALK
476	NM_015978				CARK
477	NM_018423				DKFZp761P1010
478	NM_032435	AJ311798	AX207410	AJ311797	KIAA1804, MLK4
479	AJ277481				ILK-2
480	NM_000906				NPR1
481	NM_000907	NM_003995			NPR2
482	NM_004963				GUCY2C
483	NM_000180				GUCY2D
484	NM_001522				GUCY2F
485	XM_058513	AX166563	cds: 1 - 2727		DKFZp434H2111
486					
487	NM_006218				PIK3CA (phosphoinositide-3-kinase, catalytic, alpha polypeptide)
488	NM_006219				PIK3CB (phosphoinositide-3-kinase, catalytic, beta polypeptide)
489	NM_002649				PIK3CG (phosphoinositide-3-kinase, catalytic, gamma polypeptide)
490	NM_005026				PIK3CD (phosphoinositide-3-kinase, catalytic, delta polypeptide

No.	Accession Number	Gene
491	NM_014006	SMG1
492	NM_000051	ATM (ataxia telangiectasia mutated)
493	NM_001184	ATR (ataxia telangiectasia and Rad3 related)
494	NM_014216	ITPK1
495	NM_004958	FRAP1 (FK506 binding protein 12-rapamycin associated protein 1)
496	NM_002645	PIK3C2A (phosphoinositide-3-kinase, class 2, alpha polypeptide)
497	NM_002647	PIK3C3 (phosphoinositide-3-kinase, class 3); Vps34
498	NM_002651	PIK4CB (phosphatidylinositol 4-kinase, catalytic, beta polypeptide)
499	NM_002650	PIK4CA (phosphatidylinositol 4-kinase, catalytic, alpha polypeptide)
500	NM_003496	TRRAP (transformation/transcription domain-associated protein)
501	NM_002646	PIK3C2B (phosphoinositide-3-kinase, class 2, beta polypeptide)
502	NM_004570	PIK3C2G (phosphoinositide-3-kinase, class 2, gamma polypeptide)
503	NM_006904	PRKDC (protein kinase, DNA-activated)
504	NM_013302	elongation factor-2 kinase
505	NM_025144	LAK (lymphocyte alpha-kinase)
506	NM_017662	TRPM6

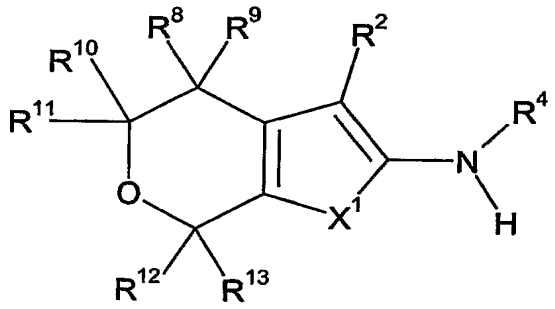
No.	Accession Number	Gene
507	NM_052947	HAK
508	NM_020778	MIDORI
509	NM_005881	BCKDK
510	NM_002610	PDK1
511	NM_002611	PDK2
512	NM_005391	PDK3
513	NM_002612	PDK4
514	NM_018343	RIOK2
515	NM_031480	RIOK1
516	NM_003831	RIOK3
517	BC017459	ADCK1
518	NM_052853	ADCK2
519	NM_020247	CABC1
520	NM_024876	ADCK4
521	NM_174922	ADCK5
522	NM_032454	STK19
523	NM_001726	BRDT
524	NM_005104	BRD2
525	NM_007371	BRD3
526	NM_058243	BRD4, var. long
527	NM_014299	BRD4, var. Short
528	NM_004606	TAF1
529	NM_153809	TAF1L

No.	Accession Number	Gene
530	NM_003852	TIF1
531	NM_005762	TRIM28
532	NM_015906	TRIM33

5

Claims

1. Compounds having the general formula (I)

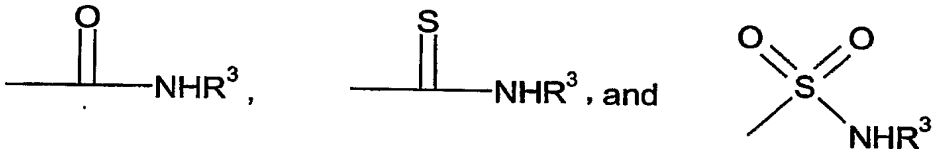


(I)

wherein

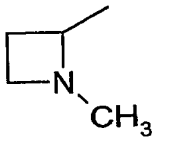
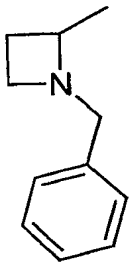
X^1 is selected from S, O, NR^1,
and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl,

R^2 is selected from



wherein R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_6-alkyl,

R^4 is selected from H , $-C(=X^2)R^5$ and $-SO_2R^5$,
wherein X^2 is O, S or NH and
R^5 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, adamantyl,


,

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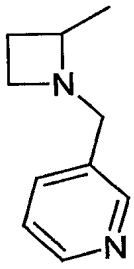
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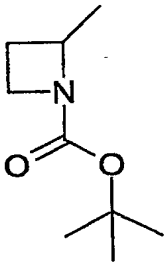
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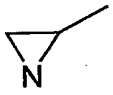
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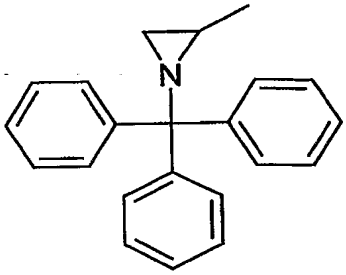


or $-(CH_2)_n-NR^{14}R^{15}$,
wherein R^{14} and R^{15} are independently selected from substituted or unsubstituted C_1-C_4-alkyl or C_2-C_4-alkenyl and wherein n = 1 to 6,
or NR^6R^7,
wherein
R^6 is selected from H, C_1-C_6-alkyl, and
R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl,

R^8 is H and R^9 is selected from H, substituted or unsubstituted C_1-C_6-alkyl
R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH
R_{11} is selected from H and substituted or unsubstituted C_1-C_6-alkyl
R_{12} is selected from H and substituted or unsubstituted C_1-C_6-alkyl, C_1-C_6-alkoxy, or OH, and
R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl,

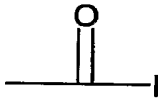
and stereoisomeric and regioisomeric forms and pharmaceutically acceptable salts of these compounds.

2. The compound according to claim 1, wherein X^1 is S.

3. The compound according to claim 1, wherein X^1 is NR^1, and R^1 is selected from H, substituted or unsubstituted C_1-C_6-alkyl, and preferably is methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, or benzyl.

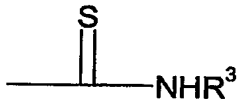
4. The compound according to claim 1, wherein X^1 is O.

5. The compound according to any one of claims 1 to 4,

wherein R^2 is  ,

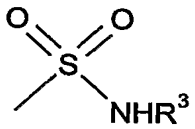
and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

6. The compound according to any one of claims 1 to 4,

wherein R^2 is  ,

and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

7. The compound according to any one of claims 1 to 4,

wherein R^2 is 

and R^3 is selected from H, HO-substituted, H_2N-substituted or HS-substituted C_1-C_4-alkyl, and preferably is H.

8. The compound according to any one of claims 1 to 7,
wherein R^3 is selected from the group consisting of H, $-CH_2-CH_2-OH$, $-CH_2-CH_2-NH_2$, $-CH_2-CH_2-SH$, $-CH_2-CH(OH)-CH_3$, $-CH_2-CH(SH)-CH_3$, or $-CH_2-CH(NH_2)-CH_3$.

9. The compound according to any one of claims 1 to 8, wherein R^4 is $-C(=X^2)R^5$ and X^2 is O or S.

10. The compound according to claim 9, wherein X^2 is O.

11. The compound according to any one of the preceding claims, wherein $R^4 = SO_2-R^5$.

12. The compound according to any one of claims 1 to 11, wherein R^5 is selected from the group consisting of substituted or unsubstituted methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, C_1-C_6 cycloalkyles substituted by at least one methyl or carboxyl group, phenyl, furanyl, thienyl, pyrrolyl, pyridyl, pyrrolidinyl, piperidinyl, tetrahydrofuranyl, ethenyl, *cis*-prop-1-enyl, *trans*-prop-1-enyl, *cis*-prop-2-enyl, *trans*-prop-2-enyl, but-1-enyl, *cis*-but-2-enyl, *trans*-but-2-enyl, but-3-enyl, prop-1-inyl, prop-2-inyl, but-1-inyl, but-2-inyl, but-3-inyl, adamantyl, or NR^6R^7, wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl.

13. The compound according to any one of claims 1 to 12, wherein R^5 is selected from the group consisting of cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, phenyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, methyl-substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, carboxyl substituted cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, furanyl, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl, *cis*- or *trans*-prop-1-enyl, but-1-enyl, adamantyl, 3,4-difluorophenyl or NR^6R^7, wherein R^6 is H and R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^7 preferably is unsubstituted cyclohexyl, phenyl, 3,4-difluorophenyl, 4-acetylphenyl, p-tolyl-phenyl or 4-fluorophenyl.

14. The compound according to claim 13, wherein R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl.

15. The compound according to claim 13 or 14, wherein R^7 is selected from substituted or unsubstituted C_3-C_6-cycloalkyl, C_1-C_6-alkyl, aryl, heteroaryl, heterocycloalkyl, C_2-C_4-alkenyl, C_2-C_4-alkinyl, or adamantyl, and R^{10} is selected from H, substituted or unsubstituted C_1-C_6-alkoxy, or OH.

16. The compound according to any one of claims 1 to 15, wherein R^8 is H and R^9 is selected from H, or substituted or unsubstituted C_1-C_6-alkyl.

17. The compound according to any one of claims 1 to 16, wherein R^8 and R^9 are both H.

18. The compound according to any one of claims 1 to 17, wherein R^{10}, R^{11}, R^{12}, and R^{13} are independently selected from H and substituted or unsubstituted C_1-C_6-alkyl, and preferably from H or methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl or tert.-butyl.

19. The compound according to any one of claims 1 to 17, wherein R^{10} and R^{11} are methyl and R^{12} and R^{13} are H or wherein R^{10}, R^{11}, R^{12}, and R^{13} are H or wherein R^{10}, R^{11}, R^{12}, and R^{13} are methyl or R^{10} and R^{11} are H and R^{12} and R^{13} are methyl.

20. The compound according to any one of claims 1 to 17, wherein R^{10} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{11} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

21. The compound according to any one of claims 1 to 17, wherein R^{12} is selected from substituted or unsubstituted C_1-C_6-alkoxy or OH and R^{13} is selected from H or substituted or unsubstituted C_1-C_6-alkyl.

22. The compound according to any one of claims 1 to 21, wherein R^1 is selected from the group consisting of methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec.-butyl, iso-butyl, tert.-butyl or benzyl.

23. The compound according to any one of claims 1 to 22, wherein R^{14} and R^{15} are independently selected from methyl, ethyl and n-propyl. iso-propyl or allyl, and preferably are methyl.

24. The compound according to anyone of claims 1 to 23, wherein the compound is selected from the group consisting of:
2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 1),
2-(Cyclopentanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 2),
2-(2-Methyl-butyrylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 3),
2-(Cyclobutanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 4),
2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 5),
2-But-2-enoylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 6),
2-(3-Methyl-but-2-enoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 7),
2-(2,2-Dimethyl-propionylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 8),
2-(3,4-Difluoro-benzoylamino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 9),
2-Isobutyrylamino-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 10),
2-[(2-Phenyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 11),
2-[(2-Methyl-cyclopropanecarbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 12),

2-[(Furan-2-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 13),
2-[(Adamantane-1-carbonyl)-amino]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 14),
2-(Cyclohexanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 15),
5,5-Dimethyl-2-(3-phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 16),
2-(Cyclopropanecarbonyl-amino)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-sulfonamide (Compound 17),
2-(3-Cyclohexyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 18),
2-(3-Phenyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 19),
2-[3-(4-Acetyl-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 20),
2-(3-p-Tolyl-ureido)-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 21),and
2-[3-(4-Fluoro-phenyl)-ureido]-4,7-dihydro-5H-thieno[2,3-c]pyran-3-carboxylic acid amide (Compound 22).

25. A compound according to claims 1 to 24 for use as a pharmaceutically active agent.

26. Use of at least one compound according to one of claims 1 to 24 as a pharmaceutically active agent.

27. Use of at least one compound according to one of claims 1 to 24 for the preparation of a medicament for the treatment of infectious diseases, including opportunistic diseases, particularly bacterially and/or virally induced infectious diseases, including opportunistic diseases.

28. Use according to claim 25 or 26, for the prophylaxis and/or treatment of infectious diseases, including opportunistic diseases, particularly bacterially and/or virally induced infectious diseases, including opportunistic diseases.

29. Use according to claim 27 or 28, wherein the bacterially induced infectious disease is one caused by a bacterium of the genus legionella.

30. Use according to claim 29, wherein the disease is legionnaires' disease.

31. Use according to claim 27 or 28, wherein the bacterially induced infectious disease is caused by a mycobacterium.

32. Use according to claim 31, wherein the mycobacterium is *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

33. Use according to claim 31 or 32, wherein the infectious disease is tuberculosis, leprosy or mycobacterially induced meningitis.

34. Use according to claim 27 or 28, wherein the virally induced infectious disease is one caused by a hepadnavirus.

35. Use according to claim 33, wherein the hepadnavirus is selected from HBV, GSHV or WHV.

36. Use of at least one compound according to one of claims 1 to 24 for the preparation of a medicament for the treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.

37. Use of at least one compound according to one of claims 1 to 24 for the prophylaxis and/or treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.

38. Use according to one of claims 36 or 37, wherein the autoimmune diseases are selected from the group comprising: asthma, chronic obstructive pulmonary diseases, systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, osteoporisis, psoriasis, inflammatory bowel disease, Crohn´s disease, ulcerative colitis, multiple sclerosis, alopecia or autoimmune diabetes mellitus.

39. Use according to one of claims 36 or 37, wherein the cardiovascular diseases are selected from the group consisting of: Adult congenital heart disease, aneurysms, angina, angina pectoris, arrhythmias, cardiovascular disease prevention, cardiomyopathies, congestive heart failure, myocardial infarction, pulmonary hypertension, hypertrophic growth, restenosis, stenosis or arteriosclerosis.

40. Use according to one of claims 36 or 37, wherein the cell proliferative disease is cancer.

41. Use according to claim 40, wherein the cancer is selected from the group consisting of: Bladder, breast, central nervous system, colon, gastric, lung, kidney, melanoma, head and neck, ovarian, cervix, glioblastoma, pancreas, prostate, stomach, skin, testis, leukaemia, Hodgkin´s lymphoma, liver and renal cancer.

42. Use according to one of claims 36 or 37, wherein said diabetes is selected from Type I diabetes or Type II diabetes.

43. Use according to one of claims 36 or 37, wherein said inflammation is mediated by cytokines, such as TNF-α, IL-1ß, GM-CSF, IL-6 and/or IL-8.

44. Use according to one of claims 36 or 37, wherein the neurodegenerative diseases are selected from the group comprising: Alzheimer´s disease, Parkinson´s disease, AIDS-related dementia, Huntington´s disease, amyotrophic lateral scelerosis, retinitis pigmentosa, spinal muscular atrophy and cerebrellar degeneration.

45. Use of a compound according to any one of claims 1 to 24 as an inhibitor for a protein kinase.

46. Use according to claim 43, wherein the protein kinase is a mycobacterial kinase.

47. Use according to claim 46, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

48. Use according to claim 47, wherein the protein kinase from *Mycobacterium tuberculosis* or *Mycobacterium leprae* is protein kinase G (PknG).

49. Use according to claim 45, wherein the protein kinase is a cellular kinase.

50. Use according to claim 49, wherein the protein kinase is selected from the group consisting of: EGFR, PDGF, c-kit, c-Src, GSK-3, CDK1 or SRPK1.

51 Use of at least one compound according to claims 1 to 24 for the preparation of a pharmaceutical composition.

52. Use according to claim 51, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of bacterially and/or virally induced infectious diseases, including opportunistic diseases.

53. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of bacterially induced infectious diseases caused by a bacterium of the genus legionella.

54. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of legionnaires' disease.

55. Use according to claim 52, wherein the bacterially induced infectious disease is caused by a mycobacterium, preferably *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

56. Use according to claim 54 or 55, wherein the infectious disease is tuberculosis, leprosy or mycobacterially induced meningitis.

57. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of virally induced infectious diseases, caused by a hepadnavirus.

58. Use according to claim 57, wherein the hepadnavirus is selected from HBV, GSHV or WHV.

59. Use according to claim 52, wherein the pharmaceutical composition is suitable for the prophylaxis and/or treatment of autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke.

60. Use according to claim 59, wherein the autoimmune diseases are selected from the group comprising: asthma, chronic obstructive pulmonary diseases, systemic lupus erythematosus, immune mediated glomerulonephritis, rheumatoid arthritis, osteoporisis, psoriasis, inflammatory bowel disease, Crohn´s disease, ulcerative colitis, multiple sclerosis, alopecia or autoimmune diabetes mellitus.

61. Use according to claim 59, wherein the cardiovascular diseases are selected from the group consisting of: Adult congenital heart disease, aneurysms, angina, angina pectoris, arrhythmias, cardiovascular disease prevention, cardiomyopathies, congestive heart failure, myocardial infarction, pulmonary hypertension, hypertrophic growth, restenosis, stenosis or arteriosclerosis.

62. Use according to claim 59, wherein the cell proliferative disease is cancer, wherein the cancer is selected from the group comprising:

Bladder, breast, central nervous system, colon, gastric, lung, kidney, melanoma, head and neck, ovarian, cervix, glioblastoma, pancreas, prostate, stomach, skin testis, leukaemia, Hodgkin´s lymphoma, liver and renal cancer.

63. Use according to claim 59, wherein said diabetes is selected from Type I diabetes or Type II diabetes.

64. Use according to claim 59, wherein said inflammation is mediated by cytokines, such as TNF-α, IL-1ß, GM-CSF, IL-6 and/or IL-8.

65. Use according to claim 59, wherein the neurodegenerative diseases are selected from the group comprising: Alzheimer´s disease, Parkinson´s disease, AIDS-related dementia, Huntington´s disease, amyotrophic lateral scelerosis, retinitis pigmentosa, spinal muscular atrophy and cerebrellar degeneration.

66. Use according to any one of claims 51 to 65, wherein the compound inhibits a protein kinase.

67. Use according to claim 66, wherein the protein kinase is a mycobacterial kinase.

68. Use according to claim 67, wherein the protein kinase is from *Mycobacterium tuberculosis* or *Mycobacterium leprae*.

69. Use according to claim 68, wherein the protein kinase from *Mycobacterium tuberculosis* or *Mycobacterium leprae* is protein kinase G (PknG).

70. Use according to claim 66, wherein the protein kinase is a cellular kinase.

71. Use according to claim 70, wherein the protein kinase is selected from the group consisting of: EGFR, PDGF, c-kit, c-Src, GSK-3, CDK1 or SRPK1.

Summary

Described are 4,7-dihydro-5H-thieno[2,3c]pyran derivatives and their analogues and pharmaceutically acceptable salts thereof, the use of these derivatives for the prophylaxis and/or treatment of mycobacteria-induced infections, opportunistic infections, autoimmune diseases, bipolar disorders, cardiovascular diseases, cell proliferative diseases, diabetes, inflammation, neurodegenerative diseases, and stroke, as well as compositions containing at least one 7-dihydro-5H-thieno[2,3c]pyran derivatives and their analogues derivative and/or pharmaceutically acceptable salts thereof.